



Trace Metal Analysis of Biological Samples using the Carbon Rod Atomizer - a Review

Application Note

Atomic Absorption

Author

Keith G. Brodie

Introduction

In modern medicine, trace elements are receiving considerable attention as the two extremes of great excess and gross deficiency continue to be recognized. The measurement of very low concentrations of trace metals, often on volumes less than 1 mL, has presented a considerable challenge to the analytical chemist. In many instances furnace atomization with atomic absorption measurement has permitted rapid and precise analysis of such samples. A classic example is the analysis of toxic metals in blood when sample volume available is strictly limited as in paediatric clinical research studies.

One of the earliest applications was in the analysis of iron, copper, lead and zinc in blood and plasma in 1971 in which the Model 61 Carbon Rod Atomizer was chosen [1]. The conclusion from this preliminary study was that the technique showed good agreement with more time consuming procedures such as solvent extraction.

Since that time Agilent carbon rod atomizers have been successfully applied to many analyses of biological samples.



Agilent Technologies

Analytical Data

Tissue Samples

A simple technique was presented for the analysis of copper in small fragments of tissue [2]. Dissolution of the tissue in concentrated nitric acid was the only step required and 2 μL volumes were dispensed into the Model 61 Carbon Rod Atomizer. Precision for a set of samples was 7.7% RSD and the recovery for copper was in the range 94–100%.

Copper deficiency in babies has been clearly associated with Menkes' kinky hair syndrome and detailed studies have been reported [3]. Serum copper, red cell copper and the copper content of various tissue samples were analyzed in an endeavour to elucidate the mechanism of the problem. Tissue was treated with nitric acid and the digested sample applied directly to the carbon rod atomizer.

For the measurement of nanogram quantities of selenium in biological digests, Ilnat found it necessary to isolate selenium from the sample matrix by a precipitation technique using ascorbic acid to produce elemental selenium [4]. Biological samples such as liver were carefully digested in a nitric-perchloric-sulfuric acid mixture and nickel was added prior to analysis (at the 5000 $\mu\text{g/mL}$ level) to improve precision.

To obtain the best results the Model 63 power supply settings were adjusted for each furnace. Typical parameters for a 5 μL sample in the graphite tube were:

Dry	5 V, 8 seconds
Ash	8 V, 5 seconds
Atomize	10 V, 1 second

Nitrogen flow had no effect on peak signal in the range 1 to 4 L/min. With each new furnace, the absorbance was found to change substantially before settling down, after about 30 firings, to a more stable response. Lifetime was about 300 firings with the tube furnace.

Manganese is involved in a number of enzyme reactions and is an essential trace element in animals. Its analysis in animal soft tissues has presented problems because of the low concentration. Furnace atomization, employing the CRA 90 and AA 375, has made this analysis possible [5].

Tissue samples (liver, heart, kidney) were homogenized in 0.2% v/v Triton X 100 and acidified with hydrochloric acid. After heating to 60 °C for 1 hour and centrifuging, 2 μL of the clear supernatant liquid was used for injection into the furnace atomizer. The CRA 90 parameters for the graphite tube were:

Dry	100 °C, 25 seconds
Ash	900 °C, 10 seconds
Atomize	2500 °C, 0.5 seconds, ramp rate 700 °/s.

For plasma samples the threaded graphite tube was preferred and 10 μL samples were injected in order to achieve the desired sensitivity. Higher temperatures were then required during ashing to remove non atomic material (1300 °C for 10 seconds). With these samples, more than 100 atomizations were possible with the one graphite tube.

A novel scheme for the determination of manganese in small samples of biological tissues was described by Belling and Jones [6]. 0.3 g of tissue was digested by boiling in 2 mL nitric acid plus 20 drops sulfuric acid. Heating was required. Interferences were removed by first extracting iron with cupferron MIBK below pH 1; the pH of the aqueous portion was raised to 7.5 and the manganese extracted with cupferron-MIBK to separate it from Na, K, Ca, Mg which were shown to interfere. After evaporation of the MIBK, the residue was dissolved in nitric acid and measured with the M63 Carbon Rod Atomizer.

Parameters were:

Dry	5 V, 60 seconds
Ash	5 V, 30 seconds
Atomize	7.5 V, 3.5 seconds

Nitrogen was used at 4 L/min to protect the graphite.

Blood and Serum

There are eleven trace metals that are regarded as essential to living organisms: Zn, Cu, Fe, Co, Mn, Mo, Sr, Se, Cr, V, and Ni [7].

At least 17 non essential elements are found in the human body in appreciable quantities, and those elements clearly regarded as toxic include As, Be, Ba, Cd, Hg, Sb and Pb.

Atomic absorption has proved to be an invaluable technique in the measurement and detection of many trace metals in body fluids.

Lead and Cadmium

The analysis of lead and cadmium has received a lot of attention because of the widespread use of these elements in our community. Kubasik and Volosin reported the analysis of lead in whole blood with the CRA 63 and AA 5 [8]. One volume of blood was simply mixed with 2 volumes of 5% v/v aqueous Triton X 100, and 1 μL of this solution applied directly to the graphite tube. Standards were also prepared in a whole blood matrix.

Results compared well with a direct injection method in which 0.5 μL of whole blood was analyzed using the same standard solutions. Background correction was necessary at the 217 nm wavelength used because of a non atomic signal equivalent to about 7 $\mu\text{g Pb}/100\text{ mL}$ of blood. Linearity of calibration extended to at least 75 $\mu\text{g Pb}/100\text{ mL}$ of blood.

Difficulty has generally been experienced in directly analyzing whole blood, largely because of the frothing which occurs during the drying stage. For this reason, small volumes (0.5 μL) are often used and consequently precision tends to suffer.

Treatment of whole blood with Triton X 100 has been widely used for the analysis of trace metals in blood. Noller and Bloom used this method in a large survey and confirmed that the technique gave accurate results when compared to other solvent extraction and acid digestion procedures [9]. They chose a 1:1 mixture of blood: 5% aqueous Triton X 100. Their recoveries of added lead ranged from 85% to 124%. Nitrogen was used as inert gas and the M 63 parameters for blood Triton samples were as follows .

Dry	5.5 V, 34 seconds
Ash	5.6 V, 25 seconds
Atomize	3 V, 5 seconds

When many blood samples have been analyzed by the Triton dilution procedure, ash tends to build up in the graphite tube and create problems [9,10,11].

However, other researchers have found that the non-atomic absorption is minimized and ash build up consequently eliminated by a blood acid digestion procedure [12,13].

Garnys and Matousek [13] mixed one 50 μL volume of whole blood with one 50 μL volume of concentrated nitric acid and heated the solution to about 85 $^{\circ}\text{C}$ for 20 minutes. This can be achieved in a steam bath. Rapid vortex mixing and light centrifuging of the 100 μL mixture were carried out before adding 2 μL of the clear liquid to the graphite tube atomizer. Standard additions are necessary for the calibration in order to overcome chemical interference effects. It has been observed that aqueous lead solutions give about twice the signal of the same lead concentration in the blood acid solution [10].

The advantage of this acid treatment was that non-atomic absorption was minimized and background correction was unnecessary when the 283.3 nm lead line was employed. (Note that despite the reduced atomic absorption signal at 283.3 nm compared with 217.0 nm, the signal:noise level is about the same.)

Brodie and Stevens used the blood acid solution procedure to measure normal levels of lead and cadmium in whole blood [10]. These solutions remain stable for some months and provide excellent sensitivity. Background correction was necessary at the cadmium wavelength (228.8 nm) but there was no background absorption coincident with the lead peak at 283.3 nm.

CRA 90 parameters used for the analysis of 2 μL volumes were as follows:

Dry	110 $^{\circ}\text{C}$, 35 seconds
Ash	*450 $^{\circ}\text{C}$, 20 seconds
Atomize	2400 $^{\circ}\text{C}$, 0 second, 400 $^{\circ}/\text{s}$ Ramp (*400 $^{\circ}\text{C}$ for cadmium)

The high atomization temperature (greater than that required for Pb and Cd atomization) ensured that there was no build up of other matrix material present in the sample. The ASD 53 Automatic Sample Dispenser was used in that study and precision ranged from about 2 %RSD to 6 %RSD.

A particular advantage of the graphite cup was exploited by a research group who analyzed lead and cadmium in blood [14]. Frothing during the dry stage resulted in sample loss unless the cup was used. The importance of a careful drying stage was recognized in this report on the analysis of lead and cadmium, for which the graphite tube and cup were used respectively. After 5 μL of whole blood was added into the graphite (tube or cup) nitric acid was added to provide an *in situ* digestion. For cadmium analysis, the inert gas was stopped during the ash phase to permit air oxidation, and minimize non atomic absorption. The standard addition technique was necessary to establish the calibration.

The accurate and precise analysis of lead in whole blood has been established by D. Hinton in a hospital pathology laboratory [15]. 50 μL of heparinized whole blood sample was mixed with an equal volume of 10% aqueous Triton X 100 and vortexed.

Standards were prepared by adding aqueous lead solutions to blood, similarly diluted 1:1 with 10% Triton X 100 and vortexed. A 2 μL volume was injected into the graphite cup and analyzed at 283 nm using background correction.

The inert gas flow of nitrogen was critical and was carefully maintained at 4 L/min.

CRA 90 parameters for this analysis on an Agilent AA 575 were:

Dry	100 °C, 60 seconds
Ash	500 °C, 30 seconds
Atomize	1800 °C, 2.5 s, ramp rate 300–400 °/s

Another proven technique for toxic metals in blood was developed by Oldfield [16].

Thoroughly mixed heparinized blood (100 μL) was treated with equal volumes of APDC solution (0.1% w/v containing about 0.2% Triton X 100) and water-saturated MIBK. After thorough vortex mixing and centrifuging, 1 μL of the MIBK solution was injected into a graphite tube. Standard additions were required for calibration (217.0 nm) and background correction was unnecessary.

The CRA 90 parameters employed for this analysis were:

Dry	100 °C, 15 seconds
Ash	800 °C, 10 seconds
Atomize	1850 °C, 0.8 s, ramp rate 300 °/s

Nitrogen flow was maintained at 4 L/min.

Gold, Aluminium, Copper, Lithium, Platinum and Zinc

Two methods have been reported for measuring gold levels in plasma [17]. Plasma was fractionated, concentrated by freeze drying and re-dissolved in water. Alternatively, the plasma was used directly. A 20 μL volume of the sample was deposited into the graphite cup atomizer of the M 63 CRA. Argon was employed as the sheath gas and good correlation was obtained (over the range 5–40 $\mu\text{g Au/L}$) with a neutron activation technique for total gold in plasma. High concentrations of NaCl can, in some fractions, result in chemical interference and the addition of ammonium nitrate was therefore desirable.

The low volume capability of furnace atomization was an important reason why the method was chosen for the study of normal copper levels in whole blood [18]. Heparinized whole blood was treated with Triton X 100 in the same manner as for lead analysis [9], and aqueous copper standards, similarly prepared with Triton X 100, were perfectly satisfactory. The average precision was 2.8 %RSD for triplicate analysis of 300 samples and the accuracy was confirmed by using alternate methods of analysis.

Nothing definite may be said about the toxicity of aluminium, for this question is still being debated. Two methods have been authenticated for the analysis of aluminium in whole blood using the M 63 CRA with a modified power supply [19]. Firstly, 2 μL of whole blood was injected directly into the graphite cup and atomized at 2500 °C. In the second method the blood was digested in nitric acid and 2 μL samples of the mixture analyzed in the graphite tube. Again the standard addition technique was necessary and argon was the preferred inert gas. It has been known for some time that argon gives about a 2 fold sensitivity improvement for aluminium, when compared with nitrogen [20].

Measurement of aluminium in serum and dialysate has been successfully achieved by Pybus using an AA 775, CRA 90 and Automatic Sample Dispenser ASD 53 [21]. The following method was used:

To 0.4 mL of serum was added 0.2 mL of 1%, v/v Triton X-100 solution plus either 0.2 mL of water or 0.2 mL of standard aluminium solution (to prepare a standard addition calibration).

The standard aluminium solutions ranged from 25 to 200 µg/L and were diluted from a stock solution of 1 g/L aluminium solution containing 1.5% v/v hydrochloric acid. The diluted standards only remain usable for about 2 days and the pH of the stock solution was important in determining this stability. With a 5 µL sample volume, the following parameters were used on the CRA 90:

Dry	110 °C, 30 seconds
Ash	1250 °C, 20 seconds
Atomize	2300 °C, 0.5 seconds, ramp rate 400 °/s

Background correction was unnecessary, and hydrogen was added to the nitrogen during ashing and atomization to minimize the ashing signal, and to enhance the sensitivity by a factor of two.

Levels of aluminium found in serum were less than 0.010 µL, and consequently great care must be taken to avoid contamination from reagents and equipment.

The M 63 CRA was used in a novel way by Castilho and Herber to measure high levels of zinc in whole blood [22,23]. After diluting blood with de-mineralized water, the sample was placed in the tube which was aligned beneath the optical path. This technique avoided high dilutions and possible contamination. Lead, cadmium and copper levels in blood were measured with the graphite tube aligned in the normal manner, after simple dilutions in water. Some nitric acid was also added for the analysis of lead and copper. The ash temperatures were critical for lead and cadmium to prevent loss of analyte, in agreement with other studies [10].

One of the earliest reports used an M 63 CRA and Agilent AA 5, in which serum was diluted 1:25 with water [24]. Standard additions were used for calibrations and a 1 µL sample injected onto the tube furnace.

Operating parameters were:

Dry	7.5 V, 20 seconds
Ash	7.2 V, 20 seconds
Atomize	6.8 V, 2 seconds

No interferences were encountered and excellent agreement with flame methods was obtained.

Since that time extensive studies have been carried out by Ehrlich and Diamond on the same subject [25,26]. The blood sample was diluted to contain 67.5 mM ammonium nitrate and 5% trichloroacetic acid, and a 2.5 µL volume of the mixture injected into the CRA 90 graphite tube furnace, and measured on the Model 1200. Standards were prepared as LiCl in the same matrix.

The operating parameters were:

Dry	95 °C, 20 seconds
Ash	500 °C, 10 seconds
Atomize	2300 °C, 2.5 seconds

Very pure nitrogen was used at 4 L/min and the built-in capability of adding hydrogen during ashing (at 1 L/min) enhanced the signal 3 fold. The graphite tube was replaced after 70 firings, and precision of the analysis ranged from 2–4 %RSD.

This method (about 4 orders of magnitude more sensitive than the conventional flame) was also successful for sodium, potassium, calcium and magnesium with slight modification. For example, ashing temperatures of 700, 700,500 and 500 °C, atomization temperatures of 1800,1900, 2300 and 2300 °C were used for sodium, potassium, calcium and magnesium, respectively.

A specialized cation exchange separation technique for extracting a platinum species from blood plasma preceded CRA 90 analysis of the element [27].

A 2 µL sample volume was subjected to the following preset program:

Dry	100 °C, 45 seconds
Ash	700 °C, 15 seconds
Atomize	2300 °C, 1 second, ramp rate 600 °/s.

A standard addition to the plasma was necessary and a precision of $\pm 2\%$ was found.

Haemodialysis – Blood Analysis

Furnace atomization has been used to study the changes in concentration of lead, manganese, chromium and aluminium in blood, during haemodialysis [28]. The CRA 90 proved entirely satisfactory for this work. Of special interest was the analysis of aluminium which was found to be evenly distributed between plasma and erythrocytes. This preliminary work indicated an increased level of plasma Zn, Mn and Al after haemodialysis.

Urine

The low levels of Chromium found during normal exposure make the analysis very difficult. An M 63 CRA has been used for the analysis of chromium in urine in a study which also examined the valence state of the element [29].

In this work a graphite tube atomizer with 10 μL of undiluted urine was subjected to the following program:

Dry	up to 130 °C (0.7 V) variable time to 120 seconds
Ash	up to 900 °C (2.4 V) 45 seconds
Atomize	2300 °C (5.1 V) 3.5 seconds

Standard additions of chromium (in 1 mL) were added to 9 mL of the sample. It was found that acids facilitated ashing but were not used in order to avoid chromium contamination. Calibration was almost linear over the range 0.1–2 $\mu\text{g Cr/L}$ and background correction was apparently not necessary.

In the same study, blood was also analyzed (diluted 1:10 with water) and a separate standard addition technique was required for each blood sample.

In another report by Routh, chromium was measured with a CRA 90 coupled to an AA 775 fitted with a background corrector [30].

It was found beneficial to use the built in facility for adding hydrogen during the ashing stage to minimize the non atomic absorption. However, little signal enhancement was observed at the flow rate of hydrogen used (0.5 L/min). The sample was acidified with nitric acid (to give a 1 N concentration) and a 20 μL sample used with the following parameters.

Dry	100 °C, 40 seconds
Ash	1000 °C, 50 seconds
Atomize	2300 °C, 1.5 seconds, ramp rate 600 °/s

A standard additions procedure was necessary and the detection limit was about 0.2 $\mu\text{g Cr/L}$. The normal level found would be about 1 $\mu\text{g Cr/L}$ and so the analysis demands the utmost of the method.

Gardiner and Ottaway employed furnace AA for the measurement of cadmium in urine [31]. This work compared different commercial atomizers including an Agilent Technologies AA 375 equipped with a CRA 90 and ASD 53 automatic sample dispenser. Background correction was necessary at the 228.8 nm wavelength.

A 5 μL volume of untreated sample was used with the threaded graphite tube and CRA 90 parameters were as follows:

Dry	95 °C, 30 seconds
Ash	400 °C, 30 seconds
Atomize	1500 °C, 2 seconds, ramp rate 200 °/s

Cadmium in urine gave a linear calibration which was about 40% less sensitive than aqueous cadmium, and consequently the standard additions technique was required to maintain accuracy.

Under the conditions stated, almost complete separation of atomic cadmium from the background peak was achieved. It is important to choose parameters which give this separation, because there is then little risk of non atomic absorption affecting the accuracy of the measurement.

A detection limit of 0.17 $\mu\text{g Cd/L}$ was found, while the range found in "normal" subjects was from this level to about 1 $\mu\text{g/L}$.

Furnace atomization has found widespread application in many demanding situations in the biological clinical research field. The reports which have been reviewed here provide some indication of the successful utilization of Agilent furnace atomizers in many research laboratories, and by many independent researchers. Perhaps one of the methods outlined here may provide the answer to your analytical problem.

References

1. J. P. Matousek and B. J. Stevens, Clin. Chem., 1971, **17**, 363 .
2. B. J. Stevens, Clin. Chem., 1972, **18**, 1379.
3. D. M. Danks et al, The Lancet, May 20, 1972, p.1100.
4. M. Ihnat, Anal. Chim. Acta., 1976, **82**, 293.
5. D. I. Paynter, Anal. Chem., 1979, **51**, 2086.
6. G. B. Belling and G. B. Jones, Anal. Chim. Acta, 1975, **80**, 279 .
7. H. A. Schroeder and A. P. Nason, Clin. Chem., 1971, **17**, 461.
8. N. P. Kubasik and M. T. Volosin, Clin. Chem., 1974, **20**, 300.
9. B. N. Noller and H. Bloom, Aust J. Med. Technol., 1975, **6**, 100 .
10. K. G. Brodie and B. J. Stevens, J. Analyt. Toxicology, 1977, **1**, 282.
11. Clinical Chemistry and Chemical Toxicology of Metals, Elsevier/North Holland, 1977, ed. S.S. Brown, page 357 (K. G. Brodie, N. K. Pradhan and B. J. Stevens).
12. V. P. Garnys and L. E. Smythe, Talanta, 1975, **22**, 881.
13. V. P. Garnys and J. P. Matousek, Clin. Chem., 1975, **21**, 891.
14. F. D. Posma, J. Balke, R. Herber and E. J. Sitvik, Anal. Chem., 1975, **47**, 834.
15. D. Hinton, Princess Margaret Hospital, Christchurch, New Zealand, (unpublished work).
16. B. Oldfield, Adelaide Childrens Hospital, Adelaide, Australia, (unpublished work).
17. As reference 11, page 301 (R. Ward, C. Danpure and D. Fyfe).
18. B. N. Noller and H. Bloom, Aust. J. Med. Technol., 1976, **7**, 22.
19. F. J. Langmyhr and D. L. Tsalev, Anal. Chim. Acta, 1977, **92**, 79.
20. K. G. Brodie and J. P. Matousek, Anal. Chem. 1971, **43**, 1557.
21. J. Pybus, Auckland Hospital, Auckland, New Zealand (unpublished work).
22. P. del Castilho and R. Herber, Anal. Chim. Acta, 1977, **94**, 269.
23. As reference 11, page 361 (P. del Castilho and R. Herber).
24. D. T. Stafford and F. Saharovici, Spectrochim Acta, 1974, **29B**, 277.
25. B. E. Ehrlich and J. M. Diamond, Biochim. Biophys. Acta., 1978, **543**, 264.
26. B. E. Ehrlich and J. M. Diamond, Am. J. Physiol, 1979, **237**, C102 .
27. S. Bannister, Y. Chang, L. Sternson and A. Repta, Clin. Chem., 1978, **24**, 877.
28. As reference 11, page 45, (B. Stevens, N. Pradhan, R. Atkins and N. Thompson).
29. H. Nomiya, M. Yororiyama and K. Nomiya, Am. Ind. Hyg. Assoc., J., 1980, **41**, 98.
30. M. W. Routh, Anal. Chem., 1980, **52**, 182.
31. P. E. Gardiner and J. M. Ottaway, Talanta, 1979, **26**, 841.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem

www.agilent.com/chem

For Research Use Only. Not for use in diagnostic procedures..

Information is subject to change without notice.

© Agilent Technologies, Inc., 1980
Printed in the USA
November 1, 2010
AA009



Agilent Technologies