Selenium Determination in Blood using Zeeman Background Correction and Palladium/Ascorbic Acid Chemical Modification

Application Note

Atomic Absorption

Author

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Introduction

The measurement of selenium in blood and other biological matrices is extremely important. Selenium is an essential element to mammals and yet in many forms is highly toxic. Sheep grazed on land deficient in selenium develop "white muscle disease" while livestock grown on selenium-rich land can be poisoned after eating Astragalus (locoweed) which concentrates selenium [1]. Determinations for selenium in feed, soil or animal blood are important analyses in this area.

Recent geographical studies of human selenium levels [2] suggested an association between low selenium intake and higher incidence of cancers. If this theory holds, the determination of selenium in human blood may become a regular test required by medical practitioners. The requirement will be a careful monitoring of human blood selenium levels to maintain an effective yet non¬toxic level of selenium. Further work is required to establish or disprove any link between low cancer occurrence and selenium intake. This will require a reliable method for the determination of selenium in blood.

The analytical problems associated with the determination of selenium in blood are well documented. The difficulties can be divided into three major classes, spectral interferences, chemical interferences and thermal pre-atomization losses. Chemical interferences are generally minimized if higher ashing temperatures can be achieved via improved thermal stability.

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Spectral Interferences

Two forms of spectral interference are known for selenium. Firstly, iron which is present in whole blood at levels of about 500 mg/L, exhibits spectral lines close enough to 196.0 nm to cause an interference. Approximately 40 iron lines [3] can be found within the region 196.0 nm ±1, with the closest two lines occurring at 196.014 and 196.32 nm. It is obvious then that the selection of smaller slit widths cannot exclude all of these lines.

A second form of spectral interference is caused by the presence of phosphates in the blood. This was noted by Saeed and Thomassen [4] who attributed the interference to P_2 absorption. These authors tested various modifiers finding a 1% nickel solution to be best They claimed other modifiers tested including palladium caused a reduction in selenium sensitivity. In later work [5], the same authors overcame the spectral interferences by separating the selenium from the phosphates, by precipitating blood proteins and then determining the selenium with a modifier.

When deuterium arc background correction systems are used these spectral interferences result in overcompensation. Large negative peaks interfering with the selenium atomic peak have been observed. Hoenig recently showed [6] that these spectral Interferences could be overcome with deuterium arc background correction, using nickel nitrate modifier and peak height measurement. Hoenig showed that by careful choice of conditions the overcompensation could be shifted to a temperature that did not interfere with the peak height measurement of selenium.

The spectral lines of interfering elements cause an overcorrection by absorbing radiation from the continuum source. The simplest solution to this problem lies in the use of Zeeman background correction. If Zeeman background correction is used with the magnetic field placed around the atomizer, background correction occurs at the exact analyte wavelength. Double beam correction quality is achieved with a single source, single beam optical instrument. The use of Zeeman background correction overcomes the problems of iron and phosphate spectral interference in the determination of selenium.

Thermal Pre-Atomization Losses

Selenium is a relatively volatile element, (boiling point 685 °C) resembling sulphur in its various forms and compounds. Measurement of selenium by graphite furnace atomic absorption spectrometry (GFAAS) in difficult matrices requires some preliminary thermal stabilization of the element. Without such stabilization selenium may be lost in numerous forms at low ashing temperatures. Table 1 [7] indicates some of the many forms of selenium that could be formed in GFAAS, with their boiling points.

Many modifiers have been tested to improve the thermal stabilization of selenium. The most popular technique has involved the addition of a nickel nitrate solution either to the sample before analysis or in the graphite furnace. Other workers [8,9] have experimented with different modifiers for selenium.

Interest in palladium and platinum as matrix modifiers has been increasing. In 1981, Weibust et al [10] showed that of 19 metals tested including nickel and copper, 15 had a thermally stabilizing effect on inorganic tellurium, while for organic tellurium only three out of nine showed an effect. Palladium and platinum were proved to be the best modifiers for both forms, providing stability to 1050 °C. Tellurium is chemically similar to selenium.

Xian-Quan Shan et al [11] studied the mechanism of this stabilizing effect for lead and bismuth, and established with X-Ray Photoelectron Spectroscopy that analyte palladium bonds were formed. The analyte is therefore stabilized at much higher ashing temperatures.

Voth-Beach applied palladium stabilization to selenium [12,13] and many other elements. Throughout the work deuterium arc background correction was used. Chemical modification with palladium alone was found to be affected by the type of sample matrix and it was determined that the form of palladium was important.

Ascorbic acid was added separately and proved to be effective in maintaining the palladium in the elemental form rather than as a salt, giving further improvement in the modification. This is in line with the work of Xian-Quan Shan [11] in indicating that a Pd-analyte bond must be formed. This improved modifier allowed a 400 °C increase in selenium ashing temperature over the conventional nickel modification technique.

Importantly, the shift to higher ashing and atomization temperatures with the palladium/reducing agent modifier, produced the effects of platform atomization from a wall atomization technique. At the higher temperatures involved, complex matrices were more readily ashed producing less background and less chemical interference during atomization. The ashing of plasma and blood was more effective with wall atomization and carbon build up was minimized.

In this paper a method is presented which overcomes the spectral interferences and thermal instability associated with selenium determination in blood. Zeeman background correction is used to overcome spectral interferences. A modified version of Voth-Beach's palladium and ascorbic acid chemical modification is used to stabilize the selenium against thermal loss.

The Zeeman Effect

In normal atomic absorption spectrometry with no background present, the analyte atoms absorb some of the hollow cathode lamp line emission as shown in Figure 1(a). When a magnetic field is applied to the analyte atoms, as with a Zeeman background correction system, the absorption profile of the analyte atom typically splits into three or more components.

In the "normal" Zeeman pattern of three components, Figure 1(b), a central π component remains unshifted while two components are shifted a few picometers either side of the original analyte wavelength. The two types of components are distinguished by their polarizations, the π component being polarized parallel to the direction of the applied magnetic field, the σ components being polarized perpendicular to the field.

This difference in polarization allows the π components to be removed by a polarizer in the instrument optics, Figure 1(c). In this condition, the hollow cathode lamp signal alone can be measured, since with the magnet on and the π component removed, there is no absorption at the analyte wavelength (assuming no background). This then is the reference measurement of the Zeeman instrument, providing double beam performance from a single beam optical instrument. When the reference measurement is subtracted from that obtained in 1(a), the analyte absorption is obtained.

With background molecular absorption present and the magnetic field off, as shown in Figure 2(a), the hollow cathode lamp signal is absorbed by both the analyte and background species. With the magnetic field on, Figure 2(b) and the π component of the analyte absorption removed by the polarizer, the hollow cathode lamp energy is absorbed by the background only, again providing the reference measurement. If 2(b) is subtracted from 2(a) the absorbance due to the analyte alone is obtained, for example, the background absorbance has been Zeeman corrected.

This is the simplest case of Zeeman effect splitting. In practice more complex splitting patterns commonly occur.

Instrumental

The Agilent SpectrAA-40 Zeeman graphite furnace was used for these studies. The furnace design is based entirely on that of the GTA-96. An electromagnet symmetrically envelopes the graphite tube and provides a peak magnetic field of 0.8 Tesla with an alternating field.

Measurement and correction of rapidly changing background signals is achieved by measurement at double the mains frequency (100 Hz for 50 Hz mains), and the use of a Polynomial Interpolation Routine.

Standard Preparation

A 100 ng/mL selenium standard was prepared fresh daily in 0.5% hydrochloric acid from a selenium stock solution in hydrochloric acid. Deionized distilled water was used for all dilutions. High concentrations of nitric acid must not be used in standard preparation so as to avoid oxidation of the palladium modifier. The standard additions method of calibration was used, and additions were prepared automatically by the programmable sample dispenser.

Sample Preparation

Whole blood samples in lithium heparin or sequestrin tubes were used. Five hundred microlitres of whole blood were mixed with two millilitres of a solution of 0.5% Triton X-100, 0.125% Antifoam B (Dow Corning Co.) and 0.25% L-Ascorbic Acid (Ajax Unilab Laboratory Reagent Grade) in a centrifuge tube. The blood samples were dispensed by adjustable pipette. After an initial short drain period, the pipette tip was allowed to drain for 5 minutes with periodic clearing. This ensured reproducible between-sample results.

The diluted sample was vortexed vigorously for 30 seconds, to produce lysis of the blood cells. The samples were then centrifuged and the clear red supernatant analyzed for selenium. Uncentrifuged samples were compared to centrifuged samples and no significant difference between selenium contents of the two types could be established. However, the presence of ruptured cells in uncentrifuged samples may affect analytical precision.

Sample Handling Notes

A biological fluid such as blood requires careful sample handling:

- Minimization of sample contamination must be achieved by minimum handling, and by the use of clean laboratory ware. Centrifuge tubes used in this work were rinsed thoroughly with tap-water and detergent, rinsed with distilled water, rinsed twice with Milli-Q water then dried under a blast of filtered air. Autosampler vials and auto-pipette tips had been soaked in dilute acid, then rinsed twice in Milli-Q deionized distilled water, air-dried, and stored in a sealed container. All reagents must be tested for contamination by running a representative blank.
- All biological fluids should be treated as a disease risk and handled only with gloves. Precautions should be taken to safely dispose of sampling by-products such as used tips, soiled tissues and vials. In this work most of the refuse was incinerated.
- Blood is difficult to dispense. When drawing blood into an auto-pipette tip, time should be allowed to ensure the complete volume is measured. Excess blood on the tip should be removed with a tissue, taking care not to affect the volume. The tip was allowed to drain into the centrifuge tube for a measured five minutes, Without such a draining period reproducibility between samples was poorer.
- Dispensing of the sample into the graphite furnace is greatly improved by the addition of the Triton X-100 detergent. Further improvement is obtained with the addition of the Antifoam B reagent which reduces sample foaming in the furnace, as illustrated in Figure 3. The rinse solution used in the autosampler contained approximately 0.05% hydrochloric acid and 0.05% Triton X-100. Note that nitric acid was avoided to eliminate the possibility of this oxidizing agent affecting the palladium modifier.

Method Development

The method presented by Voth-Beach for selenium in blood [12,13], involved the dilution of 1 volume of blood, with two volumes of 0.5% ascorbic acid and two volumes of 1000 mg/L palladium in 2% hydrochloric acid.

The method was attempted but it was found that some constituent of the added solutions caused precipitation of the blood proteins. This indicated the diluting solution was probably too acidic. Ascorbic acid added to blood did not cause this problem. The addition of the palladium solution caused the problem either through high acid content or by the presence of palladium itself. It was therefore not desirable to pre-mix the palladium solutions with the samples.

Ascorbic acid reduces palladium ions in solution to elemental palladium, so the mixing of the two solutions produces a precipitate of the metal. The precipitate formed settles in the sample vials with possible analyte loss. This indicates that it is desirable to keep the palladium part of the modifier separate from the ascorbic acid.

Attempting to inject all three fluids (blood, ascorbic acid and palladium) using the programmable sample dispenser, produced palladium precipitation, as the palladium combined with ascorbic acid residues in the tip.

The Agilent SpectrAA-40 Zeeman system permitted the separate pre-injection of the palladium solution by the programmable sample dispenser. This is followed by a capillary rinse step. In this method the blood samples are diluted with ascorbic acid and detergent agents, while the palladium modifier is pre-injected into the furnace.

Comparison of Nickel and Palladium Modifiers

A review of the GFAAS determination of metals in blood by Subramanian [14] lists tables of parameters and sample preparation techniques for the determination of selenium in blood. By far the most popular modifier is nickel nitrate solution. Ashing temperatures listed range from 300–1200 °C with nickel modification, and more than half of the methods listed use ashing temperatures greater than 1000 °C.

Figure 4 shows the results of a direct comparison of nickel and palladium modifiers. Figure 4(a) shows an overlay of peaks from 10 microlitres of 200 ng/mL selenium, comparing the modification effects of nickel and palladium at an ashing temperature of 1200 °C.

The nickel modifier was injected as 10 microlitres of a 1% nickel nitrate solution, compared to the same volume of a 500 µg/ml palladium solution with no ascorbic acid. It can be seen that the palladium modifier produces a selenium peak height of at least twice that produced by nickel modification. Blank measurements showed that there was no selenium present in the modifiers.

The performance of the nickel nitrate modifier was then tested at an ashing temperature of 900 °C in case selenium was being lost at 12000 °C. As Figure 4(b) shows, there was no improvement in the selenium signal at this lower temperature, with nickel modification.

Palladium Modifier With and Without Ascorbic Acid

As was shown in Figure 4(a) it is not necessary to have ascorbic acid present with the palladium to achieve chemical modification for selenium. Apparently enough palladium is in the correct state to provide a modifier that performs better than nickel. Figure 5 shows that the addition of ascorbic acid to a standard selenium solution with palladium modification increases the selenium peak height signal. Although palladium provides a modification effect by itself, the presence of a small amount of ascorbic acid ensures that maximum signal enhancement and analytical precision are obtained.

The amount of ascorbic acid necessary to achieve effective chemical modification was studied and it was found concentrations between 0.06% and 0.25% were effective. Concentrations higher than this range did not improve the palladium modified selenium peak.

Ashing Studies

To determine the effectiveness of palladium/ascorbic acid modification for selenium, several ashing studies were performed. In each case the furnace program involved a ramp to ash of ten seconds, and a hold at ash temperature for 20 seconds.

Figure 6 shows the effect of increasing ashing temperature upon unmodified selenium standard solutions. As is often observed, the selenium signal initially increases to a plateau between 300 and 400 °C. The signal begins to decline at higher temperatures and is approximately halved at 700 °C. The recommended maximum ashing temperature would be in the range 350–400 °C.

Figure 7 shows the effect of increasing ashing temperature upon a palladium-ascorbic acid modified selenium standard solution. Ascorbic acid was added to the standard solution, and the palladium pre-injected. The selenium solution remained thermally stable up to 1300 °C, after which a rapid loss of signal was observed. The thermal stability of the analyte in the region 1100–1300 °C has been thoroughly evaluated, and a maximum ashing temperature of 1200 °C is recommended.

Figure 8 gives the results of an ashing study on a blood standard addition signal (sample + standard) with palladium-ascorbic acid. The levels of the background and atomic absorbance are shown. Background levels rapidly decrease between 1100 and 1200 °C for real samples, while ashing at 1300 °C reduces the background only slightly more. The atomic signal increases gradually to a plateau between 1200 and 1300 °C but falls off rapidly above 1300 °C. This work confirms that the most practical ashing temperature is 1200 °C, for blood samples.

Palladium Modifier Concentration

The effect of using different amounts of palladium modifier was evaluated. The use of 10 microlitres of 500 µg/mL palladium pre-injected gave the same results as equal or higher volumes of higher concentrations. Lower concentrations of palladium did not improve results, and indicated precision may be worse at very low concentrations of palladium.

Results

The instrument status parameters are listed in Table 2. The standard additions method of calibration was used with peak height measurement. Figure 9 shows the comparison between a standard additions calibration of a blood sample and a normal calibration from aqueous standards. The standard additions calibration, Figure 9(a), has a slope of 3.88 x 10^{-3} Abs/(ng/mL), the normal calibration Figure 9(b), has a slope of 4.27 x 10^{-3} Abs(ng/mL). Since the slopes of the two calibrations are significantly different the standard additions method of calibration must be used for the blood samples.

The graphite furnace parameters presented in Table 2 give a total determination time of two minutes. Since sample preparation time is small with this method, compared to digestion or extraction methods, the total sample throughput is favourably high.

The sampler parameters in Table 2 show that a 20 microlitre sample volume was used. Lower volumes may be used if selenium levels are high. 20 microlitres of 500 µg/mL palladium modifier were automatically pre-injected by the programmable sample dispenser.

The palladium-ascorbic acid matrix modification technique relies upon the reduction of palladium by the ascorbic acid and subsequent thermal stabilization of selenium by the palladium. Oxidizing agents such as nitric acid should be avoided and a pre-atomization oxygen or air ashing step cannot be used but the palladium-ascorbic acid matrix modifications permits the use of high ashing temperatures. Samples should be observed carefully with the mirror to ensure samples dry evenly.

Carbon Build-Up

The build up of carbonaceous material in the graphite tube is a common occurrence with biological matrices. Many workers rely upon oxygen or air ashing to reduce this build up. Extensive carbon build up can interfere with the light path and can cause imprecision in dispensing. It has been found that the pre-injection of the palladium modifier significantly reduces carbon build up.

A 10 microliter volume of palladium modifier was pre-injected and the furnace program stopped. After removing the tube it could be seen that the 10 microliter volume did not spread completely to the partitions of the graphite tube. but remained centered in the tube. Carbon build up tests were then carried out on samples, with the tube being examined after ten firings. In one set of experiments the palladium was pre-injected, in the other set it was pre-mixed. As Figure 10 (a) shows carbon build up with the pre-injected modifier was restricted to areas not covered by the modifier volume. The pre-mixed samples showed carbon build up evenly spread over the length of the tube, and exhibited significantly more build up, Figure 10(b).

The 20 microlitre volume of modifier chosen for the analysis delays carbon build up significantly.

Sample Analyses

The results of a typical blood sample analysis are given in Table 3. The number of replicates used was two. Precision, given as %RSD, improves for higher numbers of replicates. The standard additions calibration graph for these results is shown in Figure 9(a). The concentration of selenium in the original blood sample was 80.5 ng/mL or 1.02 µmoles/L.

Figure 11 (a) shows a typical standard addition peak from a blood sample. At the 1200 °C ashing temperature background levels are low. Figure 11(b) shows a complete analytical blank of all reagents used. It can be seen that contamination levels are insignificant.

Results for blood selenium were found to be in the range 80–150 ng/mL for a range of undiluted blood samples (1.0–1.9 µmoles/L).

Precision

Measurements of precision based on ten replicate analyses of a sample gave a %RSD of 5.9 in absorbance readings. This compares favourably with the precisions of the techniques surveyed by Subramanian [14]. Of eight direct analysis techniques surveyed by Subramanian, the within-run precision varied from a low of 2.5% to a high of 18.2%, the average being 6.6%.

Conclusion

The method presented for the determination of selenium in blood combines Zeeman background correction and a new method for effective matrix modification. The Zeeman background correction overcomes spectral interferences associated with this determination. The matrix modification technique provides thermal stability for selenium to 1200 °C.

The method was shown to be more effective than nickel nitrate modification. The method compared favourably to methods reviewed in the literature in terms of determination time, maximum ash temperature and precision.

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Table 1. Boiling Points of Some Selenium Compounds

Form	Boiling point °C
SeO_3	180 decomposes
Se_2Cl_2	130 decomposes
$SeCl_4$	288 decomposes
$SeOCl_2$	176 decomposes

Table 2. Selenium in Blood — Instrument Parameters

PROGRAM 1 Se in BLOOD

INSTRUMENT MODE	ABSORBANCE
CALIBRATION MODE	STANDARD ADDITIONS
MEASUREMENT MODE	PEAK HEIGHT
LAMP POSITION	1
LAMP CURRENT (mA)	10
SLIT WIDTH (nm)	1.0
SLIT HEIGHT	NORMAL
WAVELENGTH (nm)	196.0
SAMPLE INTRODUCTION	SAMPLER AUTOMIXING
TIME CONSTANT	0.05
MEASUREMENT TIME (sec)	1.0
REPLICATES	2
BACKGROUND CORRECTION	ON
MAXIMUM ABSORBANCE	1.20

FURNACE PARAMETERS

STEP NO.	TEMPERATURE (C)	TIME (sec)	GAS FLOW (L/min)	GAS TYPE	READ COMMAND
1	85	5.0	3.0	NORMAL	NO
2	95	60.0	3.0	NORMAL	NO
3	300	20.0	3.0	NORMAL	NO
4	1200	15.0	3.0	NORMAL	NO
5	1200	15.0	3.0	NORMAL	NO
6	1200	1.0	0.0	NORMAL	NO
7	2700	0.8	0.0	NORMAL	YES
8	2700	1.0	0.0	NORMAL	YES
9	2700	2.0	3.0	NORMAL	NO

SAMPLER PARAMETERS

VOLUMES (uL)

	STANDARD	SAMPLE	BLANK	MODIFIER
BLANK	--	--	35	20
ADDITION 1	5	20	10	20
ADDITION 2	10	20	5	20
SAMPLE	--	20	15	20

RECALIBRATION RATE 0

MULTIPLE INJECT NO HOT INJECT NO PRE INJECT YES

LAST DRY STEP 1

Table 3. Selenium in Blood — Typical Sample Analysis

Sample	Conc ng/mL	%RSD	Mean ABS	Readings	
Blank	0.0		0.21	0.22	0.020
Addition 1	25.0	0.3	0.153	0.152	0.153
Addition 2	50.0	0.4	0.251	0.252	0.251
Sample 1	16.1	8.7	0.062	0.058	0.066

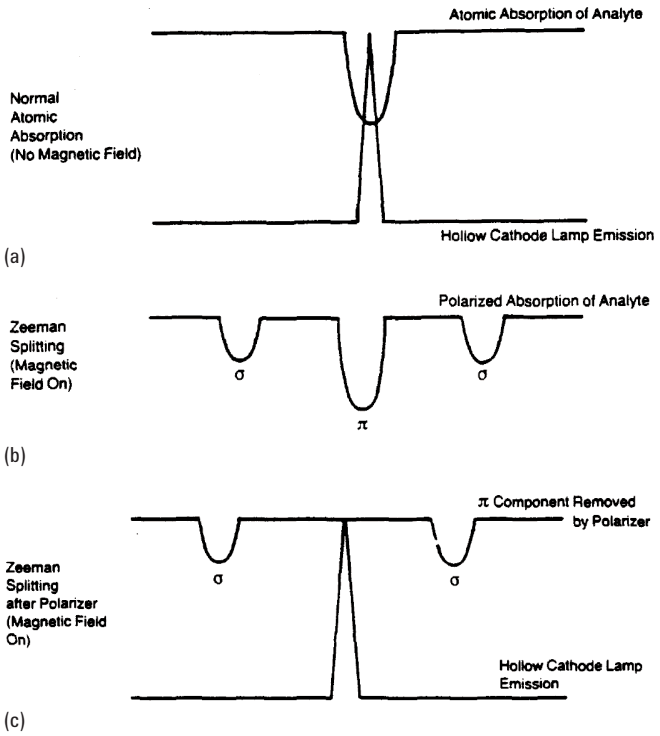


Figure 1. The "normal" Zeeman effect with no background signal.

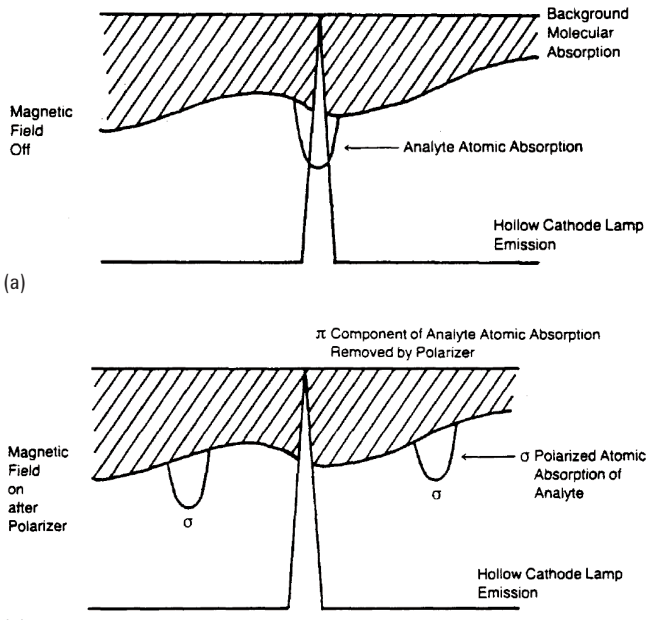


Figure 2. The "normal" Zeeman effect with background present.

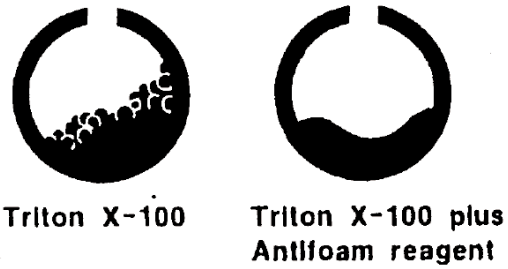
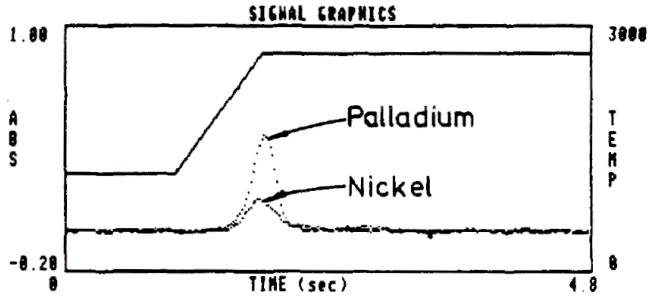
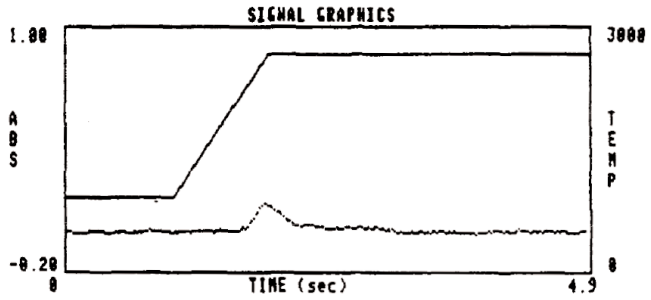


Figure 3. Whole blood dispensing.



(a) Palladium versus nickel at 1200 °C.



(b) Nickel at 900 °C.

Figure 4. Comparison of nickel and palladium chemical modifiers.

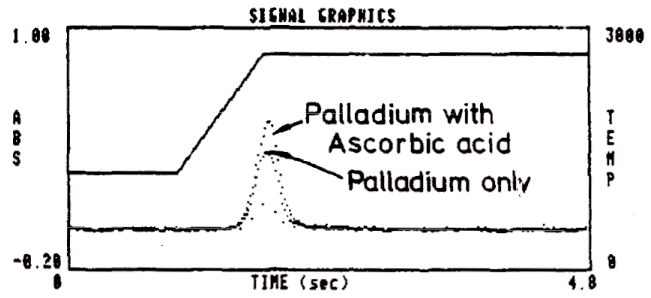


Figure 5. Comparison of palladium modification with and without ascorbic acid.

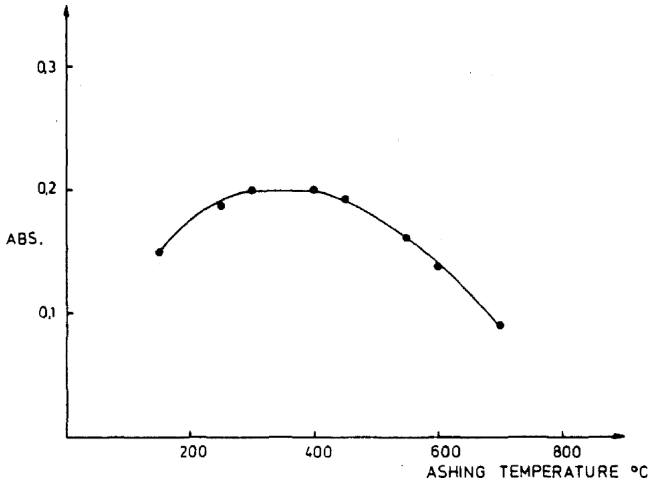


Figure 6. Ashing study — aqueous selenium solution with no chemical modifier.

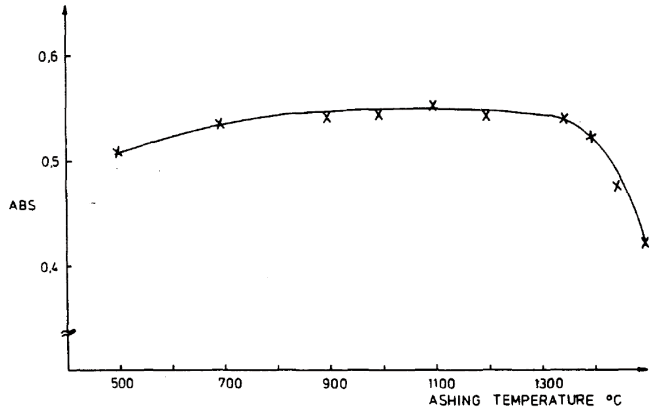


Figure 7. Ashing study — aqueous selenium solution with palladium-ascorbic acid chemical modification.

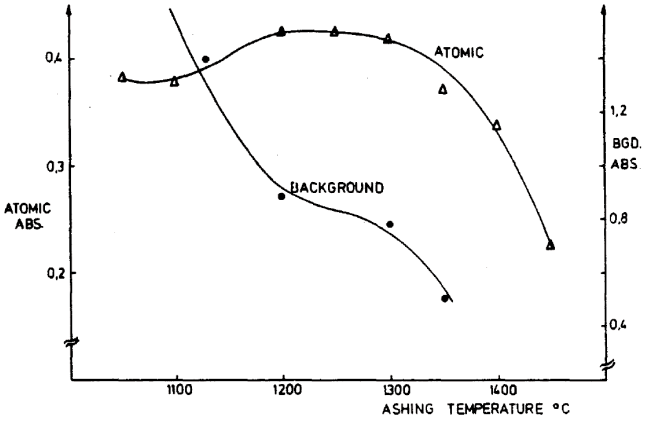
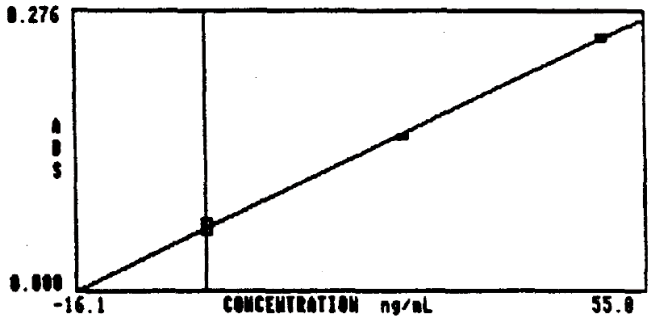
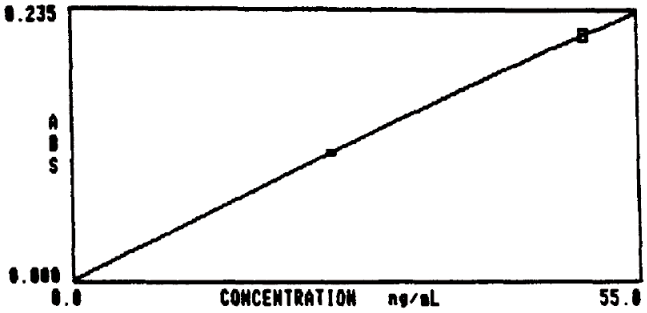


Figure 8. Ashing study — blood standard addition (sample plus standard) with palladium-ascorbic acid chemical modification.

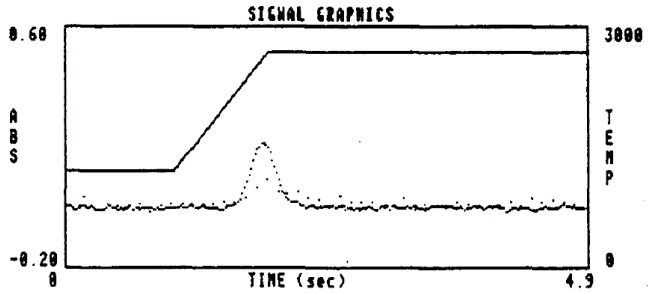


(a) Standard additions calibration.

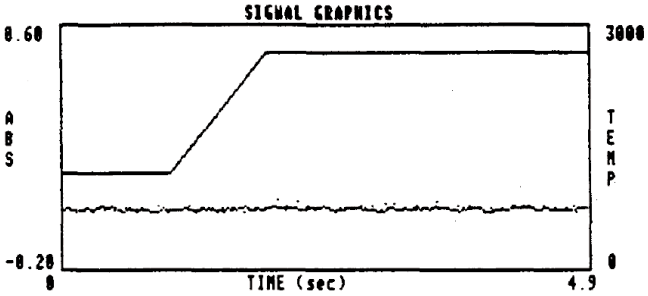


(b) Normal calibration.

Figure 9. Comparison of calibration techniques for selenium in blood.

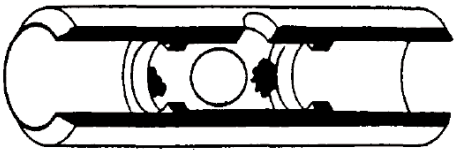


(a) Standard additions signal.

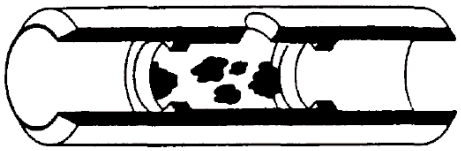


(b) Blank signal.

Figure 11. Selenium in blood — signal graphics.



(a) Palladium-ascorbic acid modifier pre-injected.



(b) Palladium-ascorbic acid modifier pre-mixed.

Figure 10. Carbon build-up experiments.

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Printed in the USA
November 1, 2010
AA070

Agilent Technologies