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## Determination of Trace Levels of Lead in Whole Blood by Graphite Furnace Atomic Absorption Spectroscopy

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DETERMINATION OF TRACE LEVELS OF LEAD IN WHOLE BLOOD BY  
GRAPHITE FURNACE  
ATOMIC ABSORPTION SPECTROSCOPY

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A Thesis

Presented to

The Faculty of the Department of Chemistry  
The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of  
Master of Arts

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by

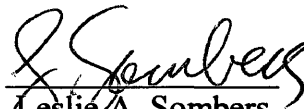
Leslie A. Sombers

1998

APPROVAL SHEET


This thesis is submitted in partial fulfillment of the requirements for the degree of

Master of Arts

  
Leslie A. Sombers

Approved, August 1998

  
Dr. Gary Rice

  
Dr. Christopher Abelt

  
Dr. William Starnes

## Dedication

This thesis is dedicated to my mother and father. They have given me the gifts of hope, persistence and optimism, and the strength to follow my dreams.

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## ABSTRACT

The purpose of this study was to develop an effective and more sensitive method for the determination of lead in blood by graphite furnace atomic absorption spectroscopy. Lead poisoning, and especially childhood lead poisoning, is a major public health problem in many industrialized countries. The presence of lead in the blood is regarded as a reliable index to the extent of recent metal uptake. Therefore, the determination of lead in blood is useful in the diagnosis of poisoning and in monitoring the working environment.

Sample pretreatment consisted of a 1:2 dilution with a dilute surfactant. To eliminate the background absorption signal from the blood matrix, a matrix modifier solution of 200 mg/L Pd and 2% citric acid in 0.01 mol/L nitric acid was also deposited onto the furnace platform. In addition, an air atmosphere was used during the pyrolysis step to aid in the combustion of matrix materials.

Values for lead in the samples were obtained by direct comparison to a linear working curve prepared from aqueous standards. An entire cycle could be completed approximately every two minutes.

The determinations were performed in triplicate, and the relative standard deviation was typically about 4% at low concentrations, indicative of very good precision. In addition, recoveries have ranged from 3 ppb to 50 ppb at low concentrations of blood lead. The limit of detection was estimated to be 0.5-1 ppb.

The method proved to be rapid and simple, reliable and was reproducible. In addition, there are minimal chances for contamination as the procedure does not require the use of many labwares and reagents. The only sample preparation is the dilution, and thus the procedure lends itself to automation. The rapidity of the method and the requirement of only tens of microliter volumes of blood sample make the method potentially attractive for large-scale screening programs.

**DETERMINATION OF TRACE LEVELS OF LEAD IN WHOLE BLOOD BY  
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## **Chapter I: Introduction**

### **Lead in the Environment**

Lead is a heavy metal of considerable utility and versatility, but is now a persistent and ubiquitous environmental problem. It has been used at least since Biblical times in a variety of products, primarily in inorganic forms to the extent that its impact as an environmental pollutant was of little consequence until this century.(1,2,3) Lead pollution has increased greatly due to its use, in metallic form, in storage batteries and in the past as organic anti-knocking additives to petrol in the automotive industry.(1,3,4,5) It is estimated that over seven million tons of lead were burned in the United States between 1926 and 1985 through internal combustion engines, and it has been well documented that high concentrations of lead are found in surface soils of even remote ecosystems as a result of atmospheric deposition.(3) Only during the last two to three decades have efforts been made to recover lead from used batteries and to use alternative petrol additives.(1,2,4,6)

The health risks associated with exposure to lead are well recognized. There is now sufficient evidence to indicate that exposure to even low lead levels (<100ug/L blood) can produce adverse health effects, especially in more vulnerable segments of the population; including the unborn fetus, infants, children and the elderly.(7) Important sources of exposure to environmental lead include paint, dust and soil in and around dwellings.(1,4,5,6)

In addition, lead in drinking water arising from lead piping, fixtures, and lead-based solder in household plumbing can also pose a significant risk.(1,4,5,6)

Chronic lead exposure is characterized by neurological defects, kidney dysfunction and anemia.(2,3,6,8) Neurological damage includes behavioral problems, intellectual impairment and hyperactivity.(1,4,8,9) Various researchers have suggested that the most controversial issue associated with the toxic effects of lead exposure is in regard to subtle behavioral effects due to moderately elevated lead levels.(1) Human behavior is influenced by so many factors that the design of studies taking these factors into account has been extremely difficult.(1)

Anatomic data suggest that the kidneys are the most sensitive to lead.(1,2,3,6) Generalized aminoaciduria is a characteristic of early lead nephropathy in both children and adults.(1,2,3,6) In children with acute exposure to lead (blood lead levels of at least 150  $\mu\text{g}/\text{dL}$ ), a complete renal Fanconi syndrome of aminoaciduria, glycosureia and hyperphosphaturia may develop.(3) The anemia of chronic lead poisoning is known to be due primarily to an inhibition of the enzyme which controls the incorporation of iron into the heme molecule.(2,3,4,6)

Overall, the current understanding of lead toxicity is considered inadequate, particularly with respect to neurobehavioral effects in man, and in defining the magnitude for maximum safe exposure levels.(1) However, in the United States the Centers for Disease Control has set the concentration of blood lead (BPb) considered harmful. The chosen value was recently lowered from 25 to 10  $\mu\text{g}/\text{dL}$  (100ppb).(10)

The assessment of lead exposures from paint, dust, soil, food and drinking water requires the reliable determination of lead in clinical samples. As a result, projects committed to the standardization of sampling, sample preparation, and analysis of lead in whole blood are a critical part of lead poisoning prevention.(7,11) The most common analytical technique for the determination of trace levels of lead in blood is through graphite furnace atomic absorption, as it requires only tens of microliters of sample and can provide detection limits up to 100 times lower than other conventional spectroscopic techniques.(12,13)

### **Comparison of Spectroscopic Analytical Methods**

A variety of analytical methods have been used for the determination of lead in biological samples. Spectroscopic techniques include: inductively coupled plasma mass spectroscopy, inductively coupled plasma atomic emission spectroscopy, and flame and graphite furnace atomic absorption spectroscopy. Each of these methods will be described briefly.

**Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).** ICP-MS is an efficient technique with low detection limits (ng/L range) and rapid sample throughput; however, instrumentation is expensive (>\$100,000). An argon plasma is used as the ion source for a quadrupole mass spectrometer.(14) Spectral interferences do not occur in the normal sense of ICP, but interferences from mass overlaps due to other elemental isotopes and

polyatomic species produced in the plasma can occur.(14) These interferences may provide erroneous results, but can be reduced with high resolution instrumentation.(14)

ICP-MS has proven to be a powerful technique for the determination of trace metals in various matrices.(13,14,15) Trace metal determination in biological matrices, however, can be very time consuming and tedious. Salts must be removed from high salt matrices before analysis due to interferences from mass overlaps.(14) Interferences may be corrected for by procedures such as principle component analysis or isotope dilution.(14) Salts are usually removed by chemical separation procedures, such as a solvent extraction procedure, which may introduce error and thus offset analytical advantages.(13,14,15)

**Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES).** ICP-AES is the most widely accepted and utilized multielement atomic spectroscopy technique, as it can detect selected elements simultaneously or sequentially.(13,14,15) By monitoring selected analytical emission wavelengths for each element, either all at once or in a programmed sequence, many elements in a given sample can be determined in one automated analysis.(13,14,15) In addition, ICP-AES has an analytical working range of up to six orders of magnitude.(13,14,15) This broad linear dynamic range enables the determination of samples with widely varying concentrations. The detection limit (about 20 µg/L) is the primary problem for the determination of lead in blood.(15) It is inadequate for routine determinations.



The determination of trace metals in biological samples by ICP-AES typically requires a separation of the analyte from the matrix because high concentrations of salts in these samples tend to clog the sample orifice of the ICP torch.(14,15) This separation is time-consuming and can create difficulties in creating analytical blanks and in controlling contamination problems.

**Flame Atomic Absorption Spectroscopy (FAAS).** FAAS is a simple, rapid and precise method for trace metal analysis; however, large sample volumes are required and the limit of detection for lead (40-50  $\mu\text{g/L}$ ) is insufficient for trace analysis.(13) The typical analytical working range is two to three orders of magnitude.(14) For the analysis of higher concentrations of lead, FAAS may be the instrument of choice. The determination of lead at  $\mu\text{g/L}$  concentrations and below requires the use of preconcentration techniques, which are very tedious and time-consuming.(13,14) Preconcentration can be accomplished by several methods such as liquid-liquid extraction with an organic phase, ion exchange preconcentration, co-precipitation, and electrochemical methods. However, any preconcentration technique is slow and prone to contamination.

**Graphite Furnace Atomic Absorption Spectroscopy (GFAAS).** By far the most advanced and highly sensitive atomization method for atomic absorption is the graphite furnace. The limit of detection for lead analysis by GFAAS is at least ten times better than FAAS; however, GFAAS has a limited analytical working range of a little over two

orders of magnitude.(13,14) A single routine GFAAS determination for a single element normally requires two to three minutes due to the multiple step temperature program required. Only small sample volumes are required, which is particularly useful in the analysis of biological samples. Matrix interferences can be controlled, in most cases, without the incorporation of extraction and preconcentration techniques. Thus, GFAAS appears to be the most useful of the existing analytical methods for the determination of trace levels of lead in small biological sample volumes; however, many GFAAS systems with low limits of detection and good precision and reproducibility require the use of the costly Zeeman background correction systems and electrodeless discharge lamps. Further improvement in the methodologies could allow for improved simplicity and sensitivity for the technique. This would make GFAAS methods more attractive for routine lead screening programs using automated systems with sufficiently low limits of detection and no sample pretreatment steps, other than simple dilution, with more accessible GFAAS instrumentation.

## Chapter II:

# Graphite Furnace Atomic Absorption Spectroscopy

### Introduction

GFAAS is a routine analytical method for the quantification of many elements in a variety of sample matrices. In order to understand this analytical technique, a brief description of the theory behind atomic absorption and of the components of the graphite furnace are necessary.

In atomic absorption, a liquid sample is typically reduced to atoms at elevated temperatures as a narrow band of electromagnetic radiation, typically in the ultraviolet through the visible region of the spectrum, is passed through the sample.(12,13,16,17) The ground state of the atom absorbs light energy of a specific wavelength and, through electronic transitions, the atom enters the excited state as illustrated in Figure 1.

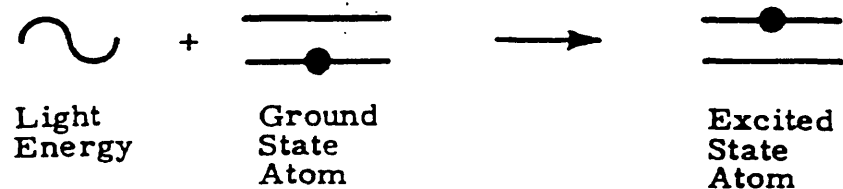


Figure 1: The process of atomic absorption. Ref.16

The amount of light absorbed is directly proportional to the number of atoms in the beam. A quantitative determination of the amount of analyte can be made by measuring the amount of light absorbed relative to known concentrations of the analyte element.(12,13,16,17) Through the use of light sources specific to the given element and carefully chosen wavelengths, the selective determination of individual elements is achieved such that spectral interferences from other species are minimized.

The intensity of the incident radiation from the radiation source ( $I_0$ ) decreases after it passes through the gaseous atoms.(12,13,16,17) The remaining intensity ( $I$ ) is given by the transmittance ( $T$ ), where

$$T = I / I_0.$$

The transmittance can then be related to the absorbance ( $A$ ) by Beer's Law, which states

$$A = - \log T = abc$$

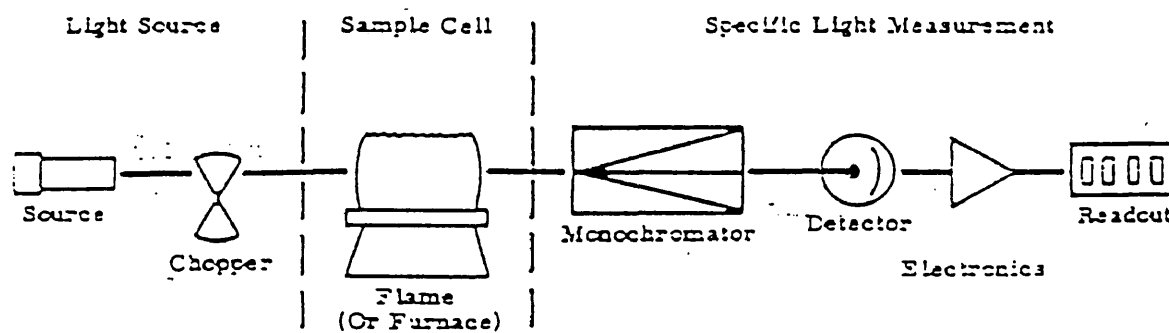
where 'a' is the absorptivity coefficient for the absorbing species, 'b' is the thickness of the sample cell (usually measured in centimeters), and 'c' is the concentration of the absorbing species. Thus, the extent of absorption depends primarily on the concentration of the absorbing species since in practice the length of the sample cell is held constant. Absorption as a function of concentration should be linear over a range of analyte concentrations measured in the same sample matrix.

At sufficiently high concentrations most elements tend to deviate from Beer's Law in that the relationship between concentration and absorbance is no longer linear. There are many reasons for this, including nonhomogeneous temperatures in the absorbing cell, stray light, line broadening and self-absorption.(12,13,16,17) In order to correct for this,

microprocessors are incorporated into modern atomic absorption instruments which plot absorbance vs. concentration curves over a wide range of concentrations. The instrument then utilizes an automatic curve correction to determine elemental content when sample concentrations are beyond the linear range.

## Instrumentation

Conventional models incorporate at least the following five instrumental components: a lamp as a source of electromagnetic radiation, a graphite tube as a sample cell in which atoms are produced, a monochromator for wavelength isolation, a detector which measures the light intensity and amplifies the signal, and a microprocessor system that will electronically interpret the signal.(12,13,16,17) A system to correct for background spectral interferences is usually incorporated as well. A schematic diagram of a typical GFAA instrument is shown in Figure 2.

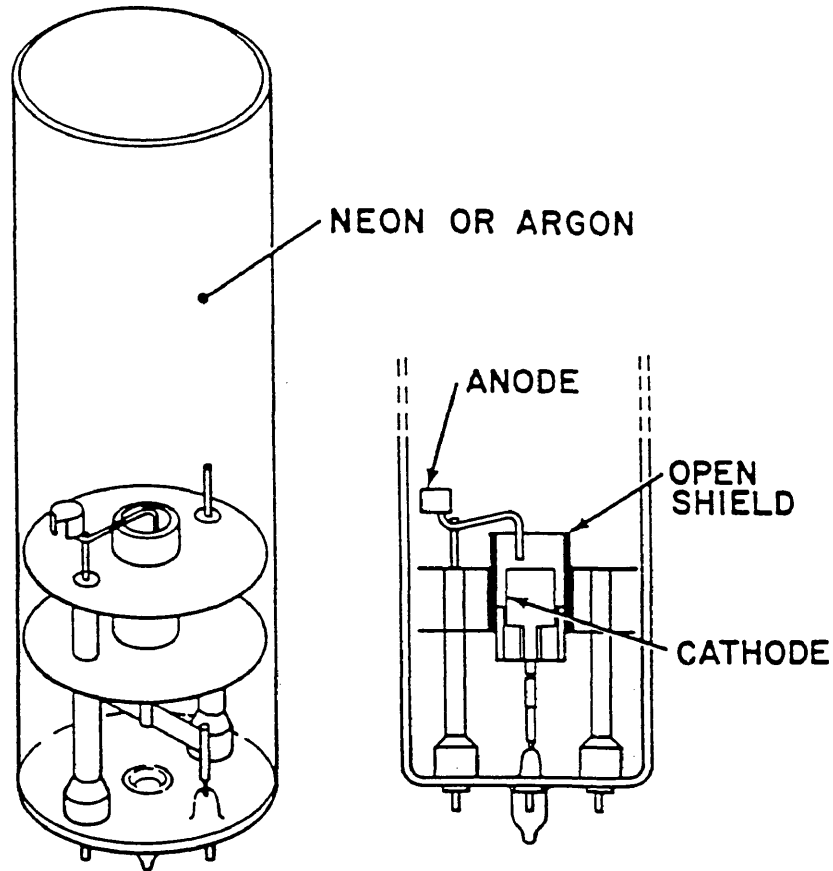


**Figure 2: Schematic diagram of atomic absorption spectrophotometer. Ref.16**

The light source that is typically used is a hollow cathode lamp (HCL), which produces a bright, stable and long-lived radiation source.(12,13,16,17) The energy required for atomization is provided by applying a high voltage electrical current through the graphite tube where the sample has been placed. A programmable power supply provides precise control of temperature programs in the atomization process. The atomic vapor generated from the furnace can absorb light. The light is electronically modulated to differentiate between the light from the source and emission from the sample cell. The monochromator is used to isolate a specific wavelength for detection by a photomultiplier tube. An electrical current is then produced and processed depending on the intensity of the light reaching the detector. A time-dependent signal is produced and either peak height or peak area can be measured and compared to a calibration curve in order to determine the analyte concentration.

Since atoms absorb light at very specific wavelengths, it is necessary to use a lamp that will provide the narrow-line spectra of the element of interest. The hollow cathode lamp is a bright, stable source for most elements.(12,13,16,17) Figure 3 illustrates how a hollow cathode lamp is constructed. The lamp consists of a tungsten anode and a cylindrical cathode sealed in a glass tube filled with neon or argon. The cathode is constructed entirely or in part of the metal whose spectrum is to be produced. Ionization of the inert gas occurs when a potential on the order of 300 V is applied across the electrodes, and a current ranging from 5 to 20 mA is generated as ions and electrons migrate to these electrodes. If the potential is sufficiently large, a process called "sputtering" occurs in which the gaseous cations acquire enough kinetic energy to dislodge some of the metal atoms from the cathode

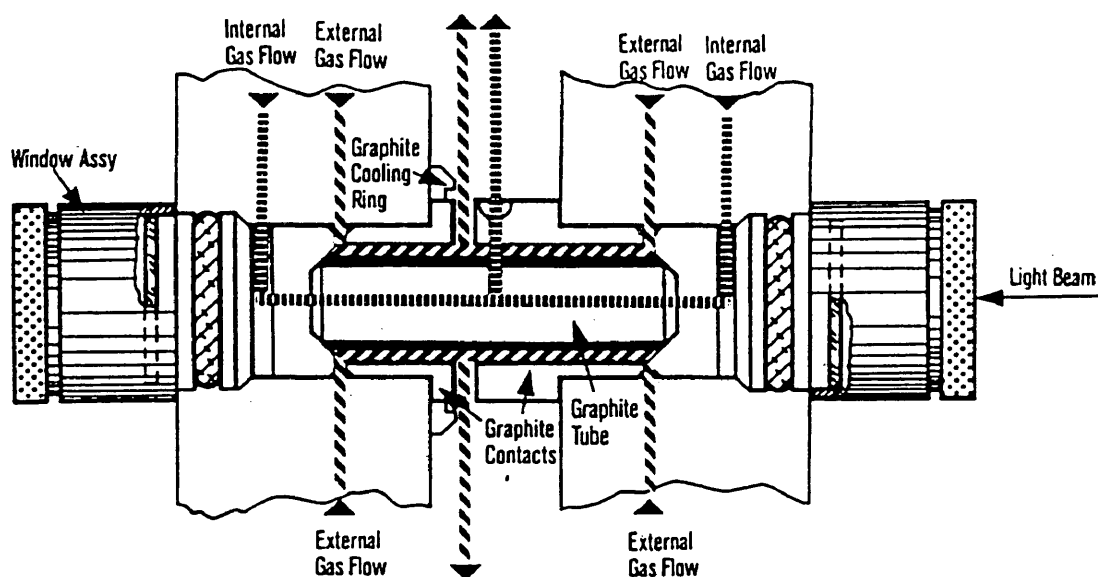
surface, thus forming an atomic cloud. These sputtered metal atoms are produced in excited states and they emit characteristic radiation in returning to the ground state. Eventually, the metal atoms are redeposited as they diffuse back to the cathode or to the glass walls.



**Figure 3: Diagram of a hollow cathode lamp. Ref.16**

The sample cell consists of an electrically heated graphite tube into which the sample is deposited.(12,13,16,17) The graphite furnace, shown in Figure 4, is heated by applying a high current across the water cooled graphite contacts to resistively heat the

graphite tube for sample volatilization. The dimensions of the graphite tube have been optimized, as the absorbance signal is dependent, among other variables, upon the residence time of the atoms in the sample tube.(10,16) Larger tubes tend to heat slowly and problematic temperature gradients along the tube length develop. Shorter tubes heat rapidly to the extent that the sample residence time would be too short.



**Figure 4: Cross-section of a graphite furnace. Ref.12**

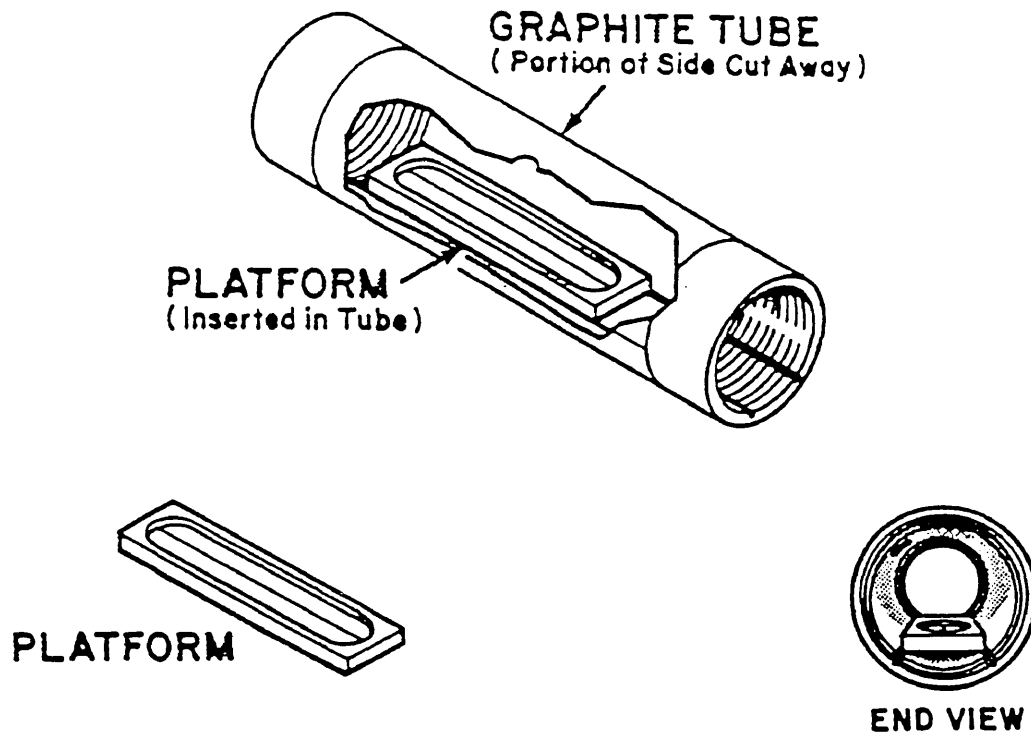
Normal graphite has a relatively coarse, layered surface which allows certain materials and elements to penetrate into the lattice where they can interact with the graphite at elevated temperatures.(12,16,17) This can pose problems because the analyte may be retained by the graphite and released only at higher temperatures. Thus, graphite tubes are commonly coated with pyrolytic graphite to make the tubes more impervious to metals in



the vapor phase. Pyrolytically coated graphite tubes have a more uniform and dense surface that does not allow for sample or solvent penetration, and they are thus more chemically inert.

When wall atomization is used, the sample is deposited onto the wall in the center of the tube.(12,16,17) This is the area of the tube that is heated most rapidly and which reaches the atomization temperature first. Thus, the analyte is volatilized off of the wall into an environment which is cooler than the surface from which it was volatilized. This leads to conditions that are very difficult to control and which can cause various matrix interferences.

In order to achieve atomization and volatilization at a more constant temperature throughout the tube, the sample is often atomized from a small platform of solid pyrolytic graphite inserted into the tube.(12,16,17) An illustration of a L'vov platform inserted in a graphite tube is shown in Figure 5. The graphite tube is heated primarily by an electric current passing through it; however, the platform is heated primarily by radiation from the tube walls. Therefore, there is a time lag between the heating of the tube and the platform, which serves to delay the atomization of the sample until the graphite tube and the inert gas reach thermal equilibrium. The L'vov platform has a proven reputation for providing greater control and reproducibility of the atomization conditions, and for reductions in sample matrix interferences.



**Figure 5: L'vov platform inserted in a graphite tube. Ref.12**

The graphite tube is completely enclosed in order to minimize environmental influences, except for the port through which the sample is deposited.(12,13,16,17) Removable quartz windows are located at both ends of the furnace to allow for passage of the HCL beam. Two independently controlled gas flows are used. An external flow of an inert gas protects the tube from ambient air exposure, which would cause degradation at high temperatures due to oxygen. Because an inert atmosphere during atomization is a prerequisite for any graphite furnace analysis, an internal gas flow is also used both before and after atomization. This gas protects the hot graphite from burning away through contact

with the ambient air and serves to purge the tube of any matrix materials volatilized during the pre-atomization dry and char steps. After atomization, the inert flow also serves to flush the tube of any remaining materials from the sample. Argon is typically used for both the external protective and the internal purge gas, as it does not react with the graphite or with analyte atoms.

Alternate gases can be used during the thermal char of samples with complex matrices, such as in the atomization of biological samples.(12,16,17) The use of air as a purge gas during the pyrolysis step can provide a much more effective char and thus a more complete removal of organic and biological materials, which may otherwise cause a carbon buildup on the inside of the tube and interfere with the analysis.

Graphite tubes typically have lifetimes of 50 to 300 determinations, depending on atomization rate, temperature, the rate of gas flow, and the composition of the sample matrix.(12,16,17) As the tube ages, analytical precision will decrease. The use of a L'vov platform typically results in a longer tube lifetime as the sample does not come into direct contact with the tube wall. In order to achieve the maximum useful tube lifetime the atomization temperature and time should be kept as low as possible. As the tube ages, the analytical peak may change in shape and the tube should typically be replaced when the absorbance value drops to about 20% below the original value.

Graphite furnace atomic absorption spectroscopy is not rapid due to the long interval between individual firings (2-3 minutes). Automatic samplers are almost a necessity for precise volume measurements and the reproducible deposition of samples into the graphite tubes. The manual deposition of a small volume of liquid into a small, dark sample opening

is very difficult and requires substantial analytical expertise. Furthermore, not only accurately dispensed volumes, but also the position of the sample droplet in the graphite tube has an influence on the precision of the results.(12,16,17)

## **Interferences**

Interferences in graphite furnace atomic absorption are evidenced by either an enhancement or depression of the absorbance signal when compared to an appropriate standard at the same concentration.(12,16,17) Various categories of interferences exist in atomic absorption; these are chemical interferences, ionization interferences, emission interferences, spectral interferences and background absorption.

Chemical interferences result when the sample contains thermally stable compounds of the element of interest which do not decompose at the prescribed furnace temperatures.(12,16,17) As a result, the number of analyte atoms in the vapor phase capable of absorbing light is reduced. These interferences can usually be overcome with the use of a higher atomization temperature, or through the use of chemical matrix modification. Increased atomization temperatures provide additional energy to break down the compounds that are stable at the lower temperature; however, more volatile elemental species may be lost in the process. Releasing agents or complexing cations can be added to preferentially react with the interferent to release the analyte at lower temperatures and indirectly remove the chemical interference.

Ionization interferences result when the furnace temperature has enough energy to cause the removal of an electron from the atom, thus creating an ion.(12,16,17) This also

reduces the number of atoms in the ground state; and thus the extent of atomic absorption is reduced. In order to minimize this type of interference, an excess of an easily ionized element can be added to both the standards and samples. Alternatively, a lower atomization temperature may be used; however, this may create other potential interferences.

The major emission interferences in graphite furnace atomic absorption spectroscopy are due to black body radiation from the furnace tube or from highly emissive species in the sample.(12,16,17) At low analyte concentrations, the atomic absorption analysis may exhibit poor precision if the emission signal falls within the spectral bandpass being used. This radiation is evidenced by increased noise or, occasionally, by baseline shifts. The emission signal is generally due to emission from the carbon tube itself; however, materials in the furnace or sample may also emit radiation. The slit width of the monochromator may be decreased in order to somewhat compensate for this interference, resulting in some loss of sensitivity.

Spectral interferences result when an absorbing wavelength of a species present in the sample falls within the bandwidth of the absorption line of the element of interest.(12,16,17) In this case, analytical results will be erroneously high as the interfering element will augment the atomic absorption signal. A smaller slit width may be used or an alternate wavelength chosen to correct for spectral interferences.

Background absorption is a common interference in atomic absorption for which there are two primary sources: light scattering by particles in the vapor phase and molecular absorption of light by molecules in the vapor phase.(12,16,17) Some samples, especially biological samples, may absorb or scatter light from the source due to the existence of

gaseous molecular species or salts in the vapor phase at the atomization temperature. In addition, the radiation from a continuum source used to measure background absorption may be absorbed by species from the matrix if they have an absorption line within the spectral bandpass being used for the analysis. Fortunately, low levels of background absorption can be distinguished from the absorption of the element of interest. The analyte element will only absorb the narrow line emitted by the source lamp; whereas background absorption is less specific and extends over a broad band of wavelengths.

The most common way to compensate for background absorption is through background correction, which subtracts non-analyte absorption signals from the total absorbance signal generated. The deuterium arc lamp continuum source background corrector, which is the most commonly used system for background correction, is designed to automatically correct for broad-band nonatomic absorption in the UV region from 195 nm to 320 nm.(12,13,16,17) The lamp, which is adjusted to equal intensity with the primary source, emits light over a broad spectrum of wavelengths instead of specific lines. The hollow cathode lamp and light from this continuum source are passed alternately through the furnace. The element being determined absorbs only the discrete lines from the hollow cathode lamp while background interferences can absorb light over the bandwidth of the slit. The overall bandwidth (typically 0.2-0.7 nm) is quite large compared to the linewidth of the atomic transition (0.001-0.0001 nm). Thus, there is negligible error in the background correction. Finally, the difference in the two beam intensities is measured electronically and used to eliminate the effect of the background absorption.

Background correction with a continuum radiation source is limited in that it only accurately corrects for background absorption that is constant in intensity over the observed spectral bandwidth.(12,16,17) When the background absorption changes with wavelength the continuum source background corrector will subtract the average background signal, which may not be identical to the actual background absorption on the resonance line and results in overcompensation. Any baseline offset in the vicinity of the absorption signal or any over-compensation of the background is a clear indication of improper background correction. In order to prevent this, other background correction systems may be used, such as Zeeman or Smith-Hjefte systems; however, these systems are far more costly.

Background absorption can also be reduced through several alternative means. Matrix modification through the addition of other species to the sample will frequently reduce the effects of background absorption. Alternatively, one can reduce the sample size by injecting less or by diluting the sample. Increasing the temperatures of the pre-atomization steps may also reduce background absorption, particularly if the background signal is being produced by a volatile organic matrix constituent. One may also increase the flow rate of the purge gas in order to decrease the residence time of the background-producing constituents in the furnace. Unfortunately, these alternatives also often result in poorer limits of detection.

## **Optimization of Analytical Methods**

In graphite furnace atomic absorption, the desolvation of the sample, dissociation from the matrix, and the generation of analyte ground state atoms occur sequentially during

the dry, char, and atomization steps respectively. Thus, it is necessary to select the individual temperatures such that each step is effectively carried out for its intended purpose.(12,16,17)

In creating the temperature program, temperature ramping allows a controlled, gradual increase in the furnace temperature instead of a strictly stepwise procedure. Stepwise procedures may be inadequate because the thermal treatment at any particular step may be incomplete; thus causing the more volatile constituents of the matrix to dissociate, melt or rapidly boil when the temperature program abruptly advances to the next step.(12,16,17) This sample spattering may lead to poor precision and loss of sample. By using temperature ramping, each of the constituents in a complex biological sample may be more effectively decomposed.

The purpose of all pre-atomization steps is to decompose and remove all matrix materials as thoroughly as possible so that the atomization of the analyte element can be carried out with minimal interferences.(12,16,17) The temperatures for these steps must be carefully chosen as they need to be long enough and the temperature high enough to completely volatilize any particulate-producing or interfering compounds. The purpose of the dry step is to evaporate low boiling liquids from the sample while the char step is used to remove as much of the matrix as possible before atomization. The physical properties of all materials and compounds present in the sample matrix largely determine the success of this separation. Thus, the thermal stability of the matrix in which the analyte element of interest resides is of major significance. The more volatile the matrix materials and the less volatile the analyte element, the easier the separation. Thus, the addition of matrix modifiers can



chemically convert the analyte to a less volatile form, or the matrix into a more volatile form.

Matrix modification has been proposed as a technique to transform elements into a more well defined compound with known properties in graphite furnace applications. This provides for the use of optimal temperature conditions for the thermal pretreatment of the matrix.(12,16,17) Higher pretreatment temperatures ensure the best separation of the analyte from accompanying materials, and thus reduces the chance of encountering background interferences. Matrix modification may also help to avoid the appearance of multiple analyte peaks in the absorption profile due to the absorption of different compounds of the analyte element present in the original sample that are volatilized at different temperatures.

The temperature selected for atomization must be high enough to completely volatilize the analyte element within a few seconds without loss of analyte.(12,16,17) The atomization time is chosen to be as short as possible while still allowing for the complete volatilization of the analyte element. If this time is too short, some of the analyte may be retained in the furnace, thus causing erroneous results for the following samples.

The external protective gas stream around the tube is normally fixed.(12,16,17) The purge gas stream, however, must be precisely controlled such that it completely removes all volatilized material from the tube without allowing it to recondense in cooler areas of the furnace. During atomization, however, the purge gas is stopped so that the analyte atoms are kept in the beam of radiation as long as possible in order to give the highest absorbance.

## **Chapter III:**

### **The Determination of Lead in Blood via GFAAS**

Among the various techniques used for the determination of lead in blood, electrothermal atomic absorption spectroscopy (ETAAS) is very popular because of the excellent sensitivity and selectivity for lead.(18) Numerous methodologies have been published for ETAAS with wide variations in atomization, sample pre-treatments and analytical methods. Most methods involve simple dilution of the blood, normally with a haemolysing agent such as Triton X-100, and then introduction into the furnace for drying, ashing, and subsequent atomization. The main problem encountered in the application of electrothermal atomization to volatile analytes in samples containing appreciable amounts of organic matter is obtaining satisfactory separation of the ash and atomization signals. This separation is necessary, even if a background correction system is employed, in order to obtain reliable results.(12,16,17) Thus, numerous groups have developed methods to remove the organic interferences either by chemical modification of the matrix or through the use of an oxygen pre-ash to aid in the combustion of interfering species.

Eaton and Holcombe published a comparative study which evaluated various matrix modifiers and procedures.(18) In particular, the roles of O<sub>2</sub> ashing, Triton X-100, HNO<sub>3</sub> and NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> were studied. The group suggested a method for the determination

of lead in human whole blood using GFAAS. As previously described, the use of temperature ramping allows for a more controlled, gradual increase in the furnace temperature; whereas a strictly stepwise procedure as performed here can cause the more volatile constituents of the matrix to dissociate, melt or rapidly boil when the temperature program abruptly advances to the next step.(12,16,17) The temperature program used in this study is provided in Table 1.

| <b>Step</b>        | <b>Furnace Temperature (°C)</b> | <b>Ramp Time (s)</b> | <b>Hold (s)</b> | <b>Internal Gas Flow (mL/min)</b> |
|--------------------|---------------------------------|----------------------|-----------------|-----------------------------------|
| <b>Dry</b>         | 100                             | 0                    | 45              | 200                               |
| <b>Char</b>        | 900                             | 0                    | 45              | 200*                              |
| <b>Atomization</b> | 2000                            | 0                    | 3-5             | 200                               |
| <b>Cool-down</b>   | 25                              | 0                    | 25              | 200                               |

\*Denotes the alternate gas, air.

**Table 1: Temperature program for the study done by Eaton and Holcombe**

The method did not utilize a continuum background correction system, thus the reduction of spectral interferences was a primary concern. The high salt concentration of blood was reduced by using red cells rather than whole blood, as previous research has clearly shown that lead tends to adsorb on the surface of red cells. Sample preparation involved centrifugation of the blood in order to isolate the whole red cells from the blood plasma. This, however, can be tedious, timely and can introduce sampling errors and contamination.

Background measurements were made with the 280.3nm line of lead.(18) Ash buildup was noted after several firings which was difficult to remove even with a high temperature burnout in the inert sheath gas. Thus, an air ashing was used to remove the carbonaceous deposits. Air, which is approximately 20% O<sub>2</sub>, was used at 2.0 L/min during the dry and first ash step of the temperature program. In the absence of air a maximum ash temperature of 500° C could be utilized without a significant loss of lead; however, in the presence of air ash temperatures of up to 950 °C were used. This temperature was sufficient to ensure combustion of the matrix without analyte loss. The group suggested that O<sub>2</sub> was adsorbed as a surface oxide on the graphite and desorbed as CO. The CO was only released at an appreciable rate at elevated temperatures of greater than 1000° C. Thus, only a few monolayers of the graphite were removed on every firing as long as an inert atmosphere existed within the furnace at temperatures greater than 1000° C.

The study also confirmed the utility of Triton X-100 in blood analysis.(18) This surfactant reduced the sample/graphite interfacial tension and allowed improved contact between the sample and the furnace walls. Without the addition of the Triton X-100, the majority of the sample was physically lost from the furnace due to frothing during the dry, and the subsequent ashing was ineffective due to poor contact with the graphite tube. Several concentrations of Triton X-100 were used, ranging from 0.1 to 5.0% (v/v). At higher concentrations, interfacial tension was so reduced that the sample flowed out of the ends of the graphite tube resulting in a loss of signal.

Many studies have indicated that the addition of low concentrations of HNO<sub>3</sub> will stabilize aqueous solutions of lead standards and prevent adsorption of the metal onto the

storage container walls. However, this study determined that significant variations in peak heights and areas caused by minor changes in acid concentrations outweighed the benefits of its use. Many researchers have also added phosphate salts, such as  $\text{NH}_4\text{H}_2\text{PO}_4$ , as matrix modifiers to facilitate the determination of lead in blood. However, the study also indicated that the addition of phosphate salts to whole blood and whole red cells resulted in considerably higher background absorbances. The addition of  $\text{NH}_4\text{H}_2\text{PO}_4$  to aqueous lead standards and blood samples also allowed for higher temperatures to acquire the absorbance peak; however, no explanation for this phenomenon was postulated.

Fernandez also developed a method for lead determinations in whole blood by GFAA in which no matrix modifiers were used, other than a simple fivefold dilution of the blood with 0.01% Triton X-100 surfactant.(19) When the samples were diluted with de-ionized water, an appreciable buildup of residue from the blood matrix was observed in the tube, making the use of simple aqueous dilution impractical. Hemolysis of the blood by dilution with the diluent eliminated this residue buildup problem. The use of various dilution ratios was investigated, and it was found that a fivefold sample dilution provided the best compromise between adequate sensitivity and low background absorption. To eliminate the nonspecific absorption signal from the blood matrix, a deuterium arc background correction system was used.

The procedure recommended by Fernandez was problematic for the same reasons as was the aforementioned procedure. The temperature program provided below in Table 2 was suggested as optimum; however, it did not include the use of temperature ramps. In

addition, the char temperature was very low, and thus may not have removed all potential interferences.

| <b>Step</b>        | <b>Furnace Temperature (°C)</b> | <b>Ramp Time (s)</b> | <b>Hold (s)</b> | <b>Internal Gas Flow (mL/min)</b> |
|--------------------|---------------------------------|----------------------|-----------------|-----------------------------------|
| <b>Dry</b>         | 100                             | 0                    | 25              | 15                                |
| <b>Char</b>        | 525                             | 0                    | 50              | 15                                |
| <b>Atomization</b> | 2300                            | 0                    | 9               | 15                                |

**Table 2: Temperature program for the study done by Fernandez**

The average recovery was studied by adding known amounts of lead nitrate to several blood samples. This recovery was typically around 98% over a concentration range of 150-1000  $\mu\text{g/L}$  with precisions ranging from 2-4%. However, this concentration range is not a realistic range to study as the Centers for Disease Control recently lowered the concentration of blood lead considered harmful to young children from 250 to 100  $\mu\text{g/L}$ . Thus, a routine method for pediatric screening must be able to accurately and precisely detect blood lead levels lower than 100  $\mu\text{g/L}$ . In addition, this method involved a centrifugation procedure to mix the diluted blood samples, which was tedious and can lead to contamination and transfer error.

Fernandez and Hilligoss developed a different method for the determination of lead in blood using the graphite furnace.(20) However, the paper was short and inconclusive, and no limit of detection was mentioned. The method involved a fivefold

dilution of blood in 0.5% Triton-X 100, matrix modification with 0.2%  $\text{NH}_4\text{H}_2\text{PO}_4$ , deuterium arc background correction and utilization of the L'vov platform.

In contrast to the findings of Eaton and Holcombe, the results of this study indicated that the use of  $\text{NH}_4\text{H}_2\text{PO}_4$  virtually eliminated any background interferences and residue buildup from the blood matrix, thus significantly extending the lifetime of the graphite tube.(10) However, this discrepancy may be due to the fact that this group utilized whole blood whereas Eaton and Holcombe used only red cells. Thus, these results indicated that phosphate salts might be of some utility in reducing salt interferences when using complex biological samples such as whole blood.

| <b>Step</b>        | <b>Furnace Temperature (°C)</b> | <b>Ramp Time (s)</b> | <b>Hold (s)</b> | <b>Internal Gas Flow (mL/min)</b> |
|--------------------|---------------------------------|----------------------|-----------------|-----------------------------------|
| <b>Dry</b>         | 130                             | 10                   | 5               | 0                                 |
| <b>Dry II</b>      | 200                             | 15                   | 20              | 0                                 |
| <b>Char</b>        | 600                             | 15                   | 45              | 0                                 |
| <b>Atomization</b> | 1700                            | 0                    | 6               | 40                                |
| <b>Cleanout</b>    | 2500                            | 1                    | 4               | 0                                 |

**Table 3: Temperature program for the study done by Fernandez and Hillgoss**

The prescribed temperature program shown in Table 3 was more typical than the one previously suggested by Fernandez, as it included the use of temperature ramps and cleanout; however, the suggested char temperature was low. In addition, the study suggested the use of an internal argon flow during atomization, but this purge gas was not used for any other step. Ordinarily, the purge gas is used to force some of the vaporized

matrix materials and burned organic residue out of the furnace prior to atomization and during the cleanout. The use of the purge gas during atomization; however, may have forced some of the lead atoms out of the furnace and attenuated the signal. The samples were also noted to rapidly clot and suspend particulate matter, which noticeably degraded both precision and accuracy.

Pruszkowska, Carnrick and Slavin also suggested a procedure for the determination of lead in blood using a combined modifier containing 0.2%  $\text{NH}_4\text{H}_2\text{PO}_4$  and 0.05%  $\text{Mg}(\text{NO})_3$ (21) In addition, 1%  $\text{HNO}_3$  was added to the solution in order to prevent the precipitation of magnesium phosphate.

This procedure involved a two step deposition of the samples onto the L'vov platform. First, a 10  $\mu\text{L}$  aliquot of the blood sample diluted 10-fold in de-ionized water containing 0.2% Triton X-100 was automatically dispensed onto the platform using an autosampler. A 5  $\mu\text{L}$  aliquot of the modifier solution was then added on top of the previously deposited samples and standards. The temperature program was typical for the determination of lead in blood, as given in Table 4.

| Step        | Furnace Temperature (°C) | Ramp Time (s) | Hold (s) | Internal Gas Flow (mL/min) |
|-------------|--------------------------|---------------|----------|----------------------------|
| Dry         | 130                      | 1             | 60       | 300                        |
| Char        | 650                      | 1             | 45       | 300                        |
| Atomization | 1700                     | 0             | 5        | 0                          |
| Cleanout    | 2600                     | 1             | 6        | 300                        |
| Cool-down   | 20                       | 1             | 20       | 300                        |

**Table 4: Temperature Program used for the study done by Pruszkowska, Carnrick and Slavin**



The use of this method, along with a magnetic Zeeman background correction system and an electrodeless discharge lamp running at 10 Watts; enabled the group to reach a sensitive limit of detection of 10  $\mu\text{g/L}$ . In addition, a 93-101% recovery was reported, and the results were in good agreement with those from the Yale-New Haven Hospital, which also analyzed the lead content of the samples. The reported precision was better than 2.5% at levels of 200  $\mu\text{g/L}$ .

One problem that was noted involved the appearance of a small, early-running peak in the blood which was perhaps due to the absorbance of lead volatilized as the chloride. In addition, this method has proven to be impractical for reproduction when used with the more accessible and less costly deuterium arc background correction system due to the appearance of high background signals and suppressed analyte signals.

Slavin and Parsons developed a different method for the determination of lead in blood via graphite furnace atomic absorption which was more rapid than any of the others previously described.(17) This method, with Zeeman background correction, again cannot be easily replicated using a deuterium arc background correction system. Whole blood was diluted 1:10 in a phosphate modifier containing 0.2%  $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.5% Triton X-100 and dilute nitric acid with sampling on a L'vov platform. Except for the small purge gas flow during atomization the temperature program was typical as shown in Table 5.

| <b>Step</b>        | <b>Furnace Temperature (°C)</b> | <b>Ramp Time (s)</b> | <b>Hold (s)</b> | <b>Internal Gas Flow (mL/min)</b> |
|--------------------|---------------------------------|----------------------|-----------------|-----------------------------------|
| <b>Dry</b>         | 175                             | 5                    | 10              | 300                               |
| <b>Dry II</b>      | 260                             | 5                    | 10              | 300                               |
| <b>Char</b>        | 900                             | 5                    | 20              | 300                               |
| <b>Atomization</b> | 1900                            | 1                    | 5               | 30                                |
| <b>Cleanout</b>    | 2500                            | 1                    | 2               | 300                               |

**Table 5: Temperature program used in the study done by Slavin and Parsons**

An entire cycle was completed every 115 seconds with standard deviations typically about 2.5 µg/L at concentrations of 100 µg/L, indicative of very good precision. The method appeared to be rugged and reliable as well as precise and accurate, as it rapidly determined concentrations down to 10 µg/L.

Hodges and Skelding described a method for the determination of lead in blood by atomic absorption spectroscopy with electrothermal atomization using a very unique matrix modification technique.(22) The method proposed a matrix modification solution comprised of 1% orthophosphoric acid, 1% HNO<sub>3</sub>, and 0.1% Triton X-100. In addition, a pre-coating of the graphite tube with molybdenum was suggested in order to most effectively minimize matrix interferences and to promote stable routine operation.

The addition of the orthophosphoric acid was effective in raising the atomization temperature of lead, thereby allowing greater time resolution of the ash and atomization processes. The lowest temperature at which atomization was detectable was reportedly increased from 630 to 840° C in the presence of the orthophosphoric acid. It was proposed that the substitution of more volatile anions by orthophosphate was beneficial in

minimizing if not eliminating the interference of halide and sulfate salts. The role of molybdenum as a coating for carbon tubes was not clear; however, it was postulated that the coating played some part in modifying the matrix so that background absorption due to phosphate salts was reduced. Overall, the molybdenum-coated tubes appeared to allow more reproducible analyte absorption signals to be obtained from the various blood samples.

The temperature program did not include the use of temperature ramps between steps or a step for the HGA cleanout. In addition, no internal purge gas was mentioned. The temperature program is shown in Table 6 below.

| <b>Step</b>        | <b>Furnace Temperature (°C)</b> | <b>Ramp Time (s)</b> | <b>Hold (s)</b> | <b>Internal Gas Flow (mL/min)</b> |
|--------------------|---------------------------------|----------------------|-----------------|-----------------------------------|
| <b>Dry</b>         | 100                             | 0                    | 60              | 0                                 |
| <b>Char</b>        | 750                             | 0                    | 30              | 0                                 |
| <b>Atomization</b> | 2500                            | 0                    | 2-5             | 0                                 |

**Table 6: Temperature Program used for the study done by Hodges and Skelding**

Over the extended study the method appeared to be reliable and it exhibited consistently good performance in regards to both precision and accuracy. The limit of detection was 50 µg/L, a value too high for the procedure to be realistically adopted for lead screening projects. The precision could probably have been improved if the samples were not manually injected. Additionally, the method involved more than one transfer of the blood sample into various flasks, the manual coating of the graphite tubes with

molybdenum, and the use of a calibration curve created by the method of standard additions. These procedures are timely and impractical for routine screening procedures.

Subramanian and Meranger suggested a rapid graphite furnace atomic absorption spectrometric procedure for the determination of lead in heparinized human whole blood using a slightly different phosphate salt for matrix modification.(7) In this method, a known aliquot of the blood sample was diluted fivefold with an aqueous solution composed of diammonium hydrogen phosphate and Triton X-100. A deuterium arc background correction system was utilized with a lead electrodeless discharge lamp to enhance the sensitivity of the technique. Nitrogen was used as the purge gas.

The most consistent results were obtained for a fivefold dilution of blood with 5 g of  $(\text{NH}_4)_2\text{HPO}_4$  and 5 mL of Triton X-100 per liter over the range of charring temperatures investigated, which was suggested as the matrix modification solution of choice for use in the determination of lead in blood. More specifically, it was postulated that the  $\text{NH}_4^+$  cations helped to minimize the matrix interferences from NaCl through the formation of the more volatile  $\text{NH}_4\text{Cl}$  species. The temperature program did not include the use of temperature ramps and a step to cleanout the HGA was not included as shown in Table 7.

| Step        | Furnace Temperature (°C) | Ramp Time (s) | Hold (s) | Internal Gas Flow (mL/min) |
|-------------|--------------------------|---------------|----------|----------------------------|
| Dry         | 100                      | 0             | 50       | 313                        |
| Char        | 700                      | 0             | 30       | 313                        |
| Atomization | 2300                     | 0             | 8        | 0                          |

**Table 7: Temperature program used for the study done by Subramanian and Meranger**

The detection limit for the proposed procedure was 10 µg/L, with precisions typically about 6%. The mean recovery of lead from spiked samples was reported to be 98.7%. The method was simple with minimal chances for contamination as it did not require the use of many labwares and reagents. The only sample preparation involved was the fivefold dilution of the blood with the matrix modification solution, and thus the procedure lended itself to automation. Finally, the method was rapid enough to complete one determination every two minutes, and required only microliter volumes of blood sample, thus making the method even more attractive for large-scale screening projects.

Granadillo, Navarro and Romero studied the behavior of lead in electrothermal absorption spectroscopy with graphite furnace atomization using palladium or phosphate-magnesium induced matrix modification in conjunction with the carbon-reducing effect achieved by the addition of citric acid.(23) The matrix modification solution contained 0.5 mg/L of palladium and 2% m/v citric acid in 0.01 mol/L nitric acid. For comparison purposes, a matrix modification mixture consisting of 0.6% m/v NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and 0.3% m/v Mg(NO<sub>3</sub>)<sub>2</sub> in 0.01 mol/L nitric acid was also used.

Under an oxygen atmosphere, the lead absorption signal for both matrix modification techniques was shifted later in time than with an inert atmosphere. These results suggested a carbon dependent mechanism for the reduction of the atomic precursor to form lead atoms with the production of CO. In addition, a simple method for the determination of lead by stabilized temperature platform furnace electrothermal atomic absorption spectroscopy in clinical samples was developed using palladium-induced matrix modification, citric acid, and an O<sub>2</sub> pyrolysis. The temperature program is given in Table 8.

| <b>Step</b>        | <b>Furnace Temperature (°C)</b> | <b>Ramp Time (s)</b> | <b>Hold (s)</b> | <b>Internal Gas Flow (mL/min)</b> |
|--------------------|---------------------------------|----------------------|-----------------|-----------------------------------|
| <b>Dry</b>         | 120                             | 5                    | 5               | 300                               |
| <b>Dry II</b>      | 250                             | 20                   | 10              | 300                               |
| <b>Char</b>        | 600                             | 30                   | 15              | 300*                              |
| <b>Atomization</b> | 2000                            | 0                    | 5               | 0                                 |
| <b>Cleanout</b>    | 2700                            | 1                    | 1               | 300                               |

\*Denotes the alternate gas, air.

**Table 8: Temperature program used for the study done by Granadillo, Navarro and Romero**

The limit of detection was 13 µg/L with recoveries ranging from 94 to 104% and a relative standard deviation of 2.2%. In addition, the method was reported to be free from interferences, and it appeared to be reliable and reproducible.

The matrix modification solutions were not combined directly with the sample specimens in order to prevent the formation of any precipitate, as this problem had been

reported with other procedures. A significant amount of a carbon-containing residue was deposited in the graphite tube when no alternate gas was used. These deposits then increased after consecutive firings until they reached a point at which the optical beam was obstructed. This problem was too significant to be corrected by the deuterium arc background correction system, thus, the air pyrolysis was utilized. This successfully prevented the build-up of the obstructing residue and uniformly dispersed the fine carbonaceous material across the platform. In addition, no significant deterioration of the graphite tube was reported because the oxygen reacted preferentially with the organic material.

The proposed method for the determination of lead in whole blood was simple and lended itself to automation, as most systems can be easily programmed to inject the sample and modifier in sequence, and to automatically switch to the alternate gas at pyrolysis. However the whole blood was diluted ten-fold and the limit of detection could be improved with lesser dilution.

Many of the aforementioned methods have taken advantage of a variety of combinations of matrix modifiers, unique temperature programs, extractions and other sample preparations to enhance the limit of detection, precision and reproducibility for the routine determination of lead in blood. The best results have tended to be associated with GFAA systems utilizing the Zeeman background correction system; however, these are high end systems not readily available in many laboratories. Furthermore, preliminary studies in our laboratory have indicated that the procedures prescribed for use with the Zeeman background correction system do not work with the more conventional

deuterium arc background correction system. In some instances a clean peak was not attainable at all, and when signals were obtained the precision and reproducibility were consistently lacking. Furthermore, there may be other modifier combinations or temperature variations which could potentially provide a reliable method for routine determinations with GFAA systems utilizing the more conventional deuterium arc background correction system. Thus, the emphasis of this project was to develop a rapid, simple, reliable and accurate method for the determination of lead in blood, using a more conventional GFAA system, with an enhanced limit of detection suitable for routine lead screenings.



## **Chapter IV:**

### **Experimental Procedures**

#### **Labware**

All glassware and Nalgene polyethylene containers were detergent washed, rinsed with deionized water, and soaked in 10% (v/v) HCl to prevent possible contamination from lead adsorption onto the container surfaces. A final rinse was then performed with semiconductor grade deionized water. All volumetric pipettes were soaked overnight in a high-density polyethylene container filled with 10% (v/v) HCl and then rinsed with tap water followed by thorough rinsings with semiconductor grade deionized water. All glassware and containers were covered or capped after washings and drying to prevent any contamination from the laboratory environment.

#### **Reagents and Standards**

Fisher “Reagent” grade HCl was used for the cleaning of the laboratory glassware. Fisher “Trace Metal” grade HNO<sub>3</sub> was used for modifier preparation and for the acid blank calibration solutions. The certified metal impurity for lead in the concentrated HNO<sub>3</sub> is 0.4 ppb.

The semiconductor grade deionized water was produced by first passing laboratory deionized water through a particulate filter and then through a semiconductor grade deionizing cartridge. A polybutylene tap was connected directly to the deionizing system to avoid contamination from the metal spigots. This system produces deionized water rated at 18 megaohms in resistance. Acid blanks and deionized water were periodically checked for contaminations.

Human blood serum and plasma, and pig blood plasma were obtained from the Sigma Chemical Company as a lyophilized powder. The samples and standards were diluted in 0.1% (v/v) Triton X-100. Human blood was drawn from Dr. Gary Rice and stored in a sealed heparinized tube. The whole blood was diluted in 0.5% (v/v) Triton X-100. "Electrophoresis Grade" Triton X-100 was obtained from Fisher Scientific and was subsequently diluted in deionized water. Triton X-100 is a nonionic surfactant with the formula  $C_8H_{17}(C_6H_4)(OCH_2CH_2)_xOH$  and an average molecular weight of 628 g/mol.

Plasma, serum and blood solutions with known lead concentrations were prepared by the method of standard additions. This was done by spiking the diluted organic matrix with a standard lead solution. The standard solution was prepared from a commercially available lead atomic absorption standard from Aldrich with a certified concentration of 1000  $\mu\text{g/mL}$ , and successive dilutions were made to create working standard solutions of 500  $\mu\text{g/L}$  and 100  $\mu\text{g/L}$ . The standards were transferred into Nalgene polyethylene bottles for storage in order to minimize surface adsorption. The blood matrix solution was spiked with the appropriate amount of standard in order to produce the desired blood lead concentration. For example, if 10 ppb blood lead concentration was desired in a blood

sample that was diluted 1:1, the solution would contain 200  $\mu\text{L}$  of blood, 200  $\mu\text{L}$  of 0.1% Triton X-100, and 4  $\mu\text{L}$  of the spike to produce a total lead concentration of 10  $\mu\text{g/L}$ .

$$\frac{(4\mu\text{L})*(500\mu\text{g/L})}{200\mu\text{L}} = 10\mu\text{g/L}$$

## Matrix Modifiers

Various matrix modifier solutions were prepared over the course of the study. A modifier solution containing 0.2% w/v  $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.5% v/v Triton X-100 and 0.2% v/v  $\text{HNO}_3$  was prepared from ammonium dihydrogen phosphate from the Aldrich Chemical Company that was reported to be 99.9999% pure. Fisher "TraceMetal Grade"  $\text{HNO}_3$  was used. Later in the study, solutions containing 0.2% w/v  $\text{NH}_4\text{H}_2\text{PO}_4$  and 0.05%  $\text{Mg}(\text{NO}_3)_2$  with and without the addition of 1% v/v  $\text{HNO}_3$  were also prepared. Magnesium nitrate hexahydrate, reported to be 99.995+% pure, from the Aldrich Chemical Company was used. Other variations in these concentrations were investigated over the course of the study in an effort to optimize modifier concentrations.

The palladium matrix modification solution was prepared from a palladium nitrate matrix modifier solution containing 0.2% Pd in 2%  $\text{HNO}_3$ , by Specpure from Alfa AESAR. Ten mL of the original solution, which contained 2000 mg/L, was diluted into the final solution by adding 84.3 mL deionized water, 5.7 mL of  $\text{HNO}_3$  and 2.0 g of citric acid. The citric acid monohydrate powder was obtained from the J.T. Baker Chemical Company, and

was reported to contain <0.3 ppm Pb. The resulting solution then contained 200 µg/mL Pd, 2% citric acid and 0.01 mol/L of nitric acid.

## **Instrumentation**

All atomic absorption determinations were made with a Perkin-Elmer Model 1100B Atomic Absorption Spectrophotometer equipped with an HGA 700 Graphite Furnace. The AS-70 Autosampler was used for all sample introductions using polycarbonate sampling cups. A Fisher single element hollow cathode lead lamp was used for the determination of lead. The default software choices for the analytical wavelength (283.4 nm) and the lamp current (10 mA) were used. The bandwidth slit height set on the monochromator was 0.7 nm low; however, a slit width of 0.2 nm low was also investigated.

A significant aspect of optimization involved variations in the temperature programs to provide the most enhanced and reproducible absorption signals. Signals were taken in triplicate with 2-6 second integration times and a zero second read delay. An internal argon gas flow of 300 mL/min was maintained throughout the graphite tube for every step of the temperature program except pyrolysis and atomization. An air flow of 300 mL/min was used for pyrolysis, and no internal gas flow was used for atomization. An example of specific instrument conditions used is provided in Table 9.

| <b>Step</b>        | <b>Furnace Temperature (°C)</b> | <b>Ramp Time (s)</b> | <b>Hold (s)</b> | <b>Internal Gas Flow (mL/min)</b> |
|--------------------|---------------------------------|----------------------|-----------------|-----------------------------------|
| <b>Dry</b>         | 150                             | 20                   | 20              | 300                               |
| <b>Char</b>        | 1000                            | 15                   | 30              | 300*                              |
| <b>Atomization</b> | 1650                            | 0                    | 10              | 0                                 |
| <b>Cleanout</b>    | 2700                            | 1                    | 7               | 300                               |

\* Denotes the alternate gas, air.

**Table 9: Example of a specific temperature program**

Four point calibration curves were generated from aqueous and plasma standards using optimized temperature programs. Auto-zero (absorbance set to zero) was measured using 1% HNO<sub>3</sub> with a deuterium arc continuum background correction system.

### **Sample Preparation**

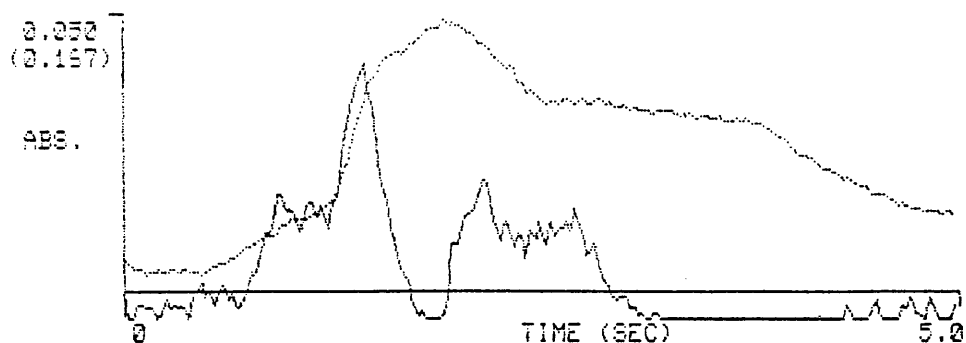
The serum and plasma samples were obtained in a lyophilized powder form and reconstituted with 2.0 mL of deionized water. All serum and plasma samples were kept refrigerated until used and discarded when clotting was evident. The whole blood sample was kept at room temperature. Test portions of the sample and standard blood, plasma and serum solutions were prepared fresh prior to each series of runs by diluting the samples to ratios (ranging from 1:1 to 1:10) in dilute Triton X-100. They were thoroughly mixed by pumping the pipettes used in transferring them to the sample cups for the AA autosampler.

## **Chapter V:**

### **Results and Discussion**

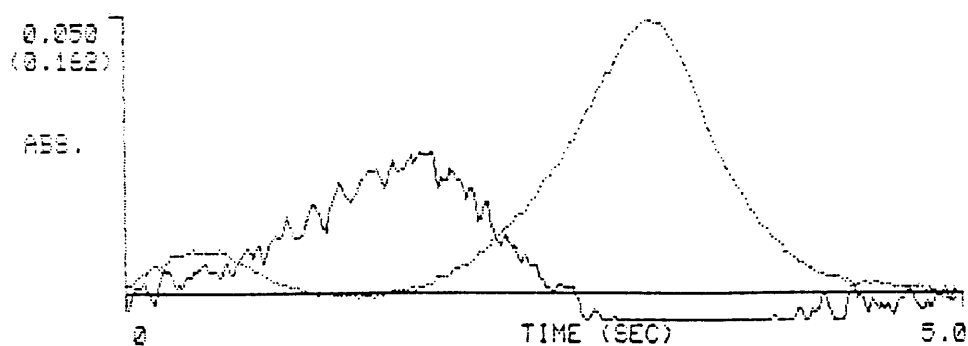
This project began with an attempt to reproduce the method used by Parsons and Slavin (10); however, human blood serum was used instead of whole blood since the primary interferences in blood were suspected to be associated with salts in the blood plasma. A matrix modifier solution containing 0.2% w/v  $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.5% v/v Triton X-100 and 0.2% v/v  $\text{HNO}_3$  was made in accordance with the method, and temperature programs for the furnace were used as recommended. The primary difference in instrumentation was the use of a continuum source background correction system instead of the Zeeman background correction system used by Slavin.

The first problem encountered was the occurrence of double peaks and signal suppression on all of the lead, an example of which is shown in Figure 6. The double peaks may have occurred because the matrix modifier solution was deposited onto the platform and then the diluted serum sample was automatically deposited on top to limit the time spent on sample loading. This may have resulted in inadequate mixing and in the formation of lead species with different volatilities at the 1900°C atomization temperature used.



**Figure 6: Double lead peaks observed using the Slavin method**

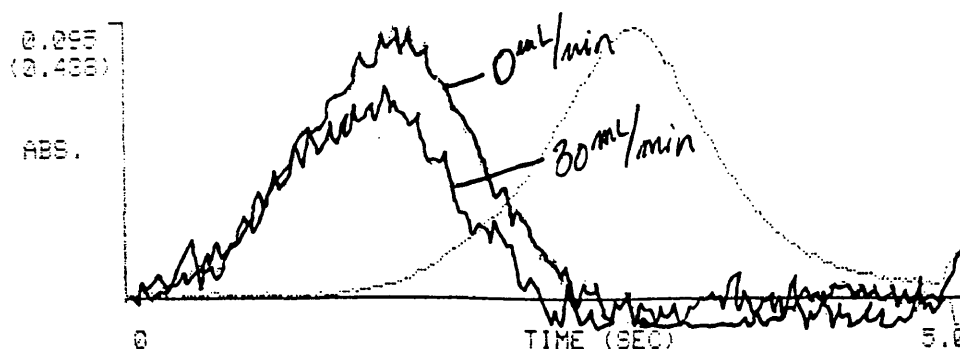
Parsons and Slavin diluted the blood samples directly with the matrix modification solution and mixed the samples well with Eppendorf pipettes. When this technique was used the double peaks disappeared; however, signal quenching due to the matrix of the blood serum was encountered. The background may have been sufficiently high to cause the baseline and signal to be suppressed as shown in Figure 7. Although the signal is apparent, it is prematurely quenched by background overcompensation. This may have been due to the instrumentation, since Parsons and Slavin utilized the Zeeman background correction system which can compensate for background signals more effectively.



**Figure 7: Overcompensation of background signal observed using the Slavin method**

In an attempt to remove the matrix interferences causing the high background, the ashing temperature was held for 25 seconds instead of the 10 seconds recommended by Parsons and Slavin. A significant improvement in the signal was detected, probably because more of the interfering species were removed prior to atomization. Lower rates of inert gas flow were tried during the atomization step using a blood serum sample spiked to 5 ppb with an aqueous lead standard. At a 30 mL/min flow, as recommended by Parsons and Slavin, the average absorbance peak area obtained over three 15  $\mu$ L sample injections was 0.025 A-s. This average steadily increased to a maximum of 0.085 A-s as the inert gas flow was lowered stepwise to 0 mL/min. Although the purge gas flow was forcing out some of the matrix interferences, it was also forcing out lead atoms during the atomization step and thus attenuating the signal. Examples of typical absorption profiles are shown in Figure 8.





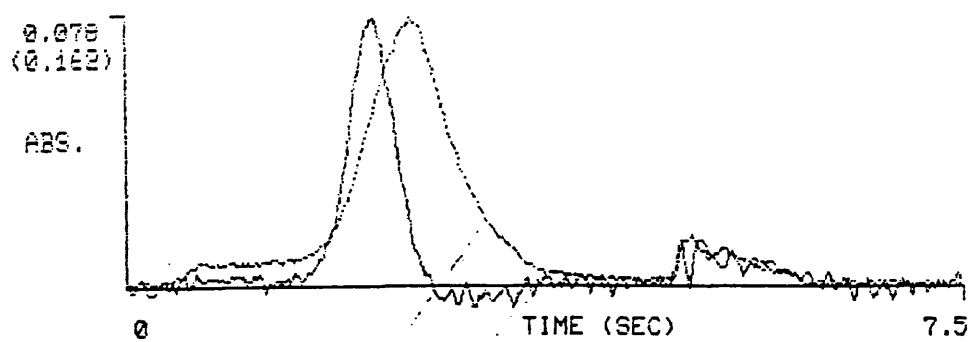
**Figure 8: Absorbance profiles for 5 ppb spiked serum at 30 mL/min and at 0 mL/min purge gas flow during atomization**

In order to improve reproducibility, a new modifier solution was made containing the original mix of chemicals without the addition of nitric acid, as recommended by Eaton and Holcombe.(18) This did in fact result in an improved reproducibility; however, signals from the blood serum samples still were attenuated relative to the aqueous lead standards.

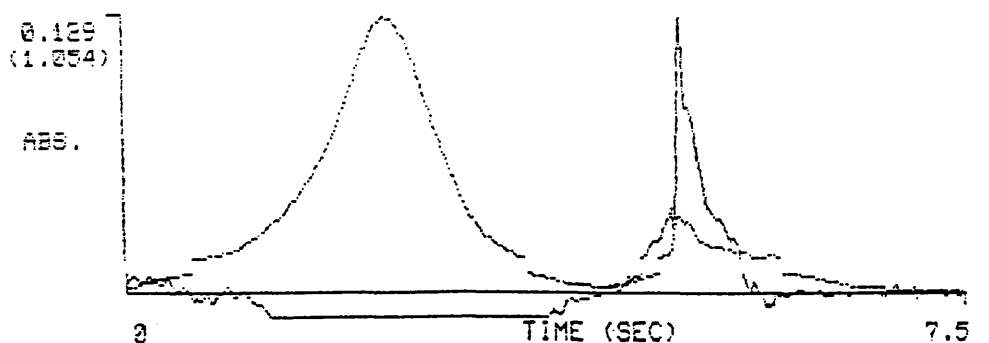
In addition, a major attenuation in the signal was observed as the sample aliquots of a 25 ppb aqueous standard, made up in the dilute modifier solution containing 0.2% w/v  $\text{NH}_4\text{H}_2\text{PO}_4$  and 0.5% Triton X-100, were increased from 15  $\mu\text{L}$  to 50  $\mu\text{L}$  for deposit in the furnace. This certainly should not have occurred as there were no salts or other species present in the aqueous standards to interfere with the signal. Visual inspection of the deposit of the sample on the platform revealed that the Triton X-100 surfactant was reducing the surface tension in the aqueous standards to the extent that the larger volumes were rolling right off of the L'vov platform, which is capable of holding up to a 50  $\mu\text{L}$

maximum aliquot size. Aqueous aliquots over 25  $\mu\text{L}$  repeatedly rolled off of the platform, resulting in a loss of signal and reproducibility. Unfortunately, the 25  $\mu\text{L}$  volume limit could not be surpassed using a different surfactant because Triton X-100 is universally recommended for blood samples to prevent clotting and precipitation.

Although Parsons and Slavin recommended a sample dilution of 1:10 with the modifier solution, the limit of detection could be improved significantly if reproducible signals could be obtained from serum samples with a lesser dilution. Thus, one serum sample was diluted 1:10 with the 0.2% w/v  $\text{NH}_4\text{H}_2\text{PO}_4$  and 0.5% v/v Triton X-100 matrix modification solution and spiked to 5 ppb, and another was diluted 1:1 with the same modifier solution and also spiked to a 5 ppb lead concentration. The signals for the samples diluted 1:10 were well formed and produced a reproducible signal with little attenuation as shown in Figure 9. The samples diluted 1:1 were very poor with no reproducibility as shown in Figure 10. The background signal was markedly higher and beyond the limits of instrumental compensation.



**Figure 9: Absorbance profile for 5 ppb spiked serum diluted 1:10, 0.2%  $\text{NH}_4\text{H}_2\text{PO}_4$  and 0.5% Triton X-100 modifier solution**



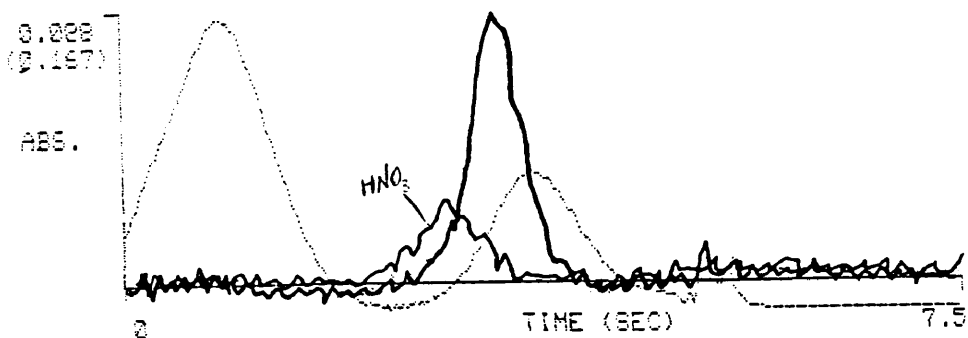
**Figure 10: Absorbance profile for 5 ppb spiked serum diluted 1:1, 0.2%  $\text{NH}_4\text{H}_2\text{PO}_4$  and 0.5% Triton X-100 modifier solution**

A new approach was undertaken to minimize the dilution ratio required. The modifier solution prescribed by Pruszkowska, Carnrick and Slavin was prepared in a manner similar to the previously tested solution in that it contained 0.2%  $\text{NH}_4\text{H}_2\text{PO}_4$  with the addition of 0.05%  $\text{Mg}(\text{NO}_3)_2$  diluted in 1%  $\text{HNO}_3$ . Pruszkowska et. al. noted that the nitric acid was added in order to prevent the precipitation of magnesium phosphate. A solution not containing the nitric acid was also prepared. No precipitation problems were detected.

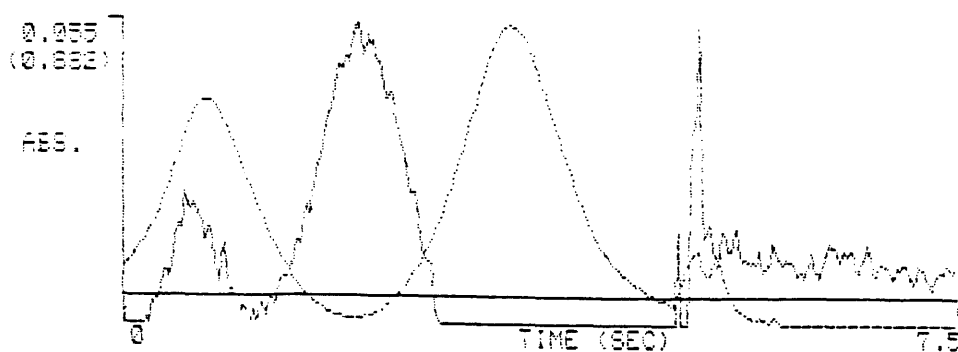
Pruszkowska, Carnrick and Slavin recommended a lower char temperature of  $650^\circ\text{C}$ , as that temperature was sufficiently high to eliminate the background interferences in their findings. Zeeman background correction was again used. It was anticipated that a higher char temperature would be necessary in this study, as the deuterium arc background correction system is not as efficient as the Zeeman. Thus, char temperatures of  $650^\circ\text{C}$  and  $900^\circ\text{C}$  were tested with both the acidified modifier solution

and the one without, using serum samples diluted 1:10 and 1:1 with 0.2% Triton X-100 and spiked with lead to 5 ppb.

First, the signal produced with the modifier solution containing 0.2%  $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.05%  $\text{Mg}(\text{NO}_3)_2$  and 1%  $\text{HNO}_3$  was compared with the solution with no acid. The 650°C char temperature was used with the sample diluted 1:10. Both sets of signals proved to be reproducible; however, the signal produced from the sample modified with the  $\text{HNO}_3$ -containing modifier consistently appeared earlier and with a much lower peak height than that produced from the sample modified with no  $\text{HNO}_3$ , as shown in Figure 11. When these conditions were tried with the serum sample diluted 1:1, the results for both char temperatures and both modifier solutions were very erratic and not at all reproducible, as shown in Figure 12. Once again the signal suppression caused by the large background and chemical interferences from the serum constituents was substantial.

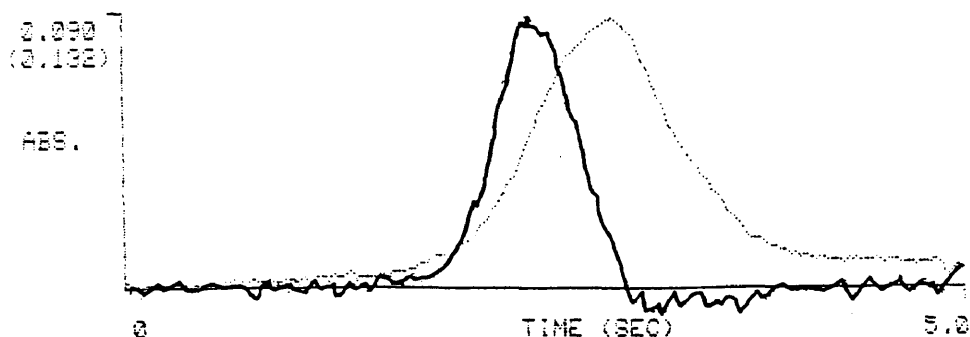


**Figure 11: Comparison of peak profiles for spiked serum diluted 1:10 using 650°C char temperature and modifiers with and without  $\text{HNO}_3$**



**Figure 12: Absorbance profile for spiked serum diluted 1:1 using 650°C char temperature and HNO<sub>3</sub>-containing modifier**

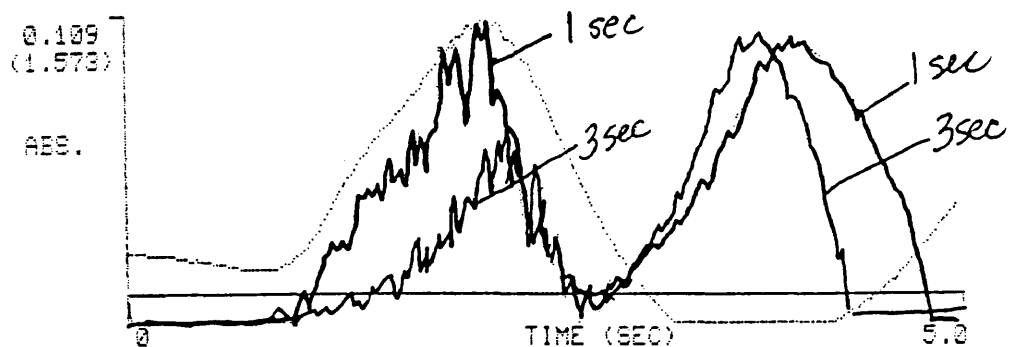
Next, both modification solutions were tried with both sample dilutions using the 900°C char temperature. As was expected, the signals for the samples diluted 1:10 were higher with the higher char temperature, as more of the matrix interferences were burned out of the sample prior to atomization. The signals from both modifier combinations were identical. A signal produced from the acidless modifier is shown in Figure 13. The signal profiles were similar to those obtained at the 650°C char, and repeated runs proved the technique to be reproducible when using the modifier without the nitric acid. Thus, it was determined that the higher char temperature was more effective when using this type of procedure. The signals for the samples diluted 1:1, however, were essentially unchanged from those obtained when using the 650°C char.



**Figure 13: Peak profile for spiked serum diluted 1:10 using 900°C char temperature and HNO<sub>3</sub>-less modifier**

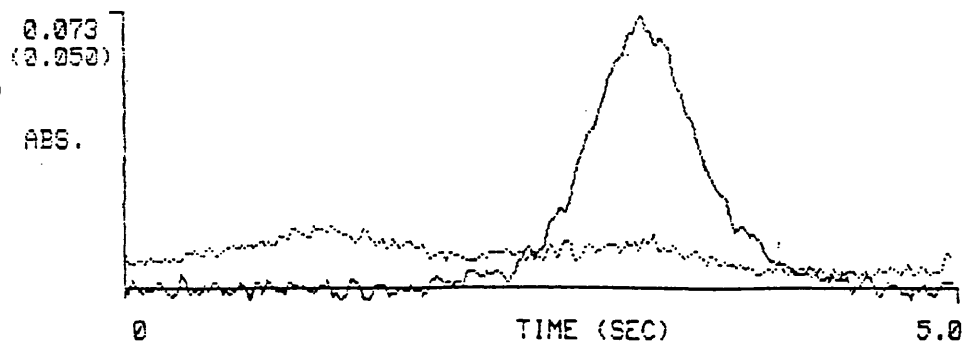
An atomization ramp time was added to the temperature program prescribed by Pruszkowska, Carrick and Slavin. Samples were diluted 1:1 directly with the acidless modifier solution with an increase to 0.5% Triton X-100 in addition to the original 0.2% NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> and 0.05% Mg(NO<sub>3</sub>)<sub>2</sub> modifiers. Initially, the char temperature of 650°C was used with the atomization temperature of 1700°C; however, the resulting 5 ppb signals were split and were very erratic regardless of the atomization ramp time selected. When the 900°C char temperature was tested, however, the results were more decipherable but two peaks were always present when an atomization ramp was included in the temperature program. Furthermore, as the atomization ramp time was increased, the area of the first peak steadily decreased and the second peak steadily appeared earlier in time. Figure 14 shows a comparison of the peak profiles when the sample was atomized at a temperature of 1700°C with a ramp of one second and with a ramp of three seconds.

Once again, there appears to be more than one lead species present with varying volatilities after the charring stage.



**Figure 14: Comparison of profiles for spiked serum diluted 1:1 with an atomization ramp of 1 and 3 sec**

The signals were then compared to the signal of an aqueous standard that was diluted 1:1 with the same modifier solution (less the Triton X-100) and spiked with lead to 5 ppb. It was discovered that the second peak of the serum sample signal closely matched the signal profile of the aqueous standard with respect to time of elution as depicted in Figure 15. Thus, it was assumed that the second peak of the serum sample signal was representative of the lead species normally produced, and that the first peak was due to a more volatile secondary species produced in the char from unknown reactions or interactions.



**Figure 15: Aqueous standard with an atomization ramp of 3 seconds**

Table 10 contains a summary of the instrumental parameters and matrix modifiers used at this stage of the investigation. The goal was to further modify the instrumental parameters in order to eliminate the first peak while keeping the sample dilution ratio at 1:1.



| Step        | Furnace Temperature (°C) | Ramp Time (s) | Hold (s) | Internal Gas Flow (mL/min) |
|-------------|--------------------------|---------------|----------|----------------------------|
| Dry         | 130                      | 5             | 45       | 300                        |
| Dry II      | 260                      | 5             | 10       | 300                        |
| Char        | 900                      | 1             | 5        | 300                        |
| Atomization | 1900                     | 3             | 5        | 0                          |
| Cleanout    | 2500                     | 1             | 2        | 300                        |

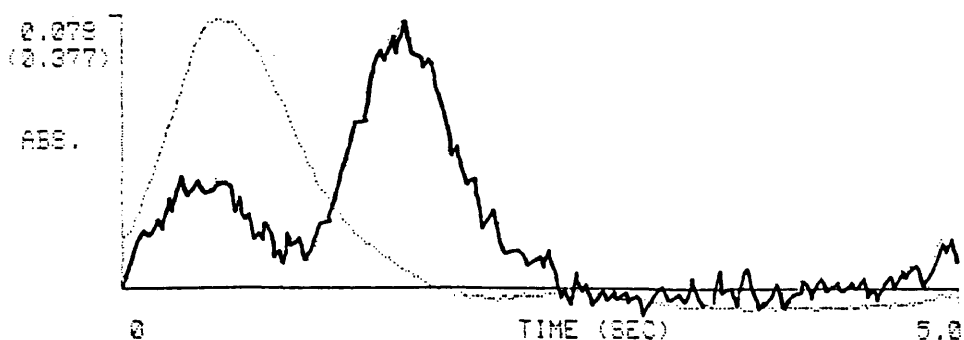
**Modifier Solution:** 0.2%  $\text{NH}_4\text{H}_2\text{PO}_4$   
0.5%  $\text{Mg}(\text{NO}_3)_2$   
0.5% Triton X-100

**Table 10: Revised procedural conditions**

As suggested by Granadillo, Navarro and Romero, the slit bandpass through which the light sources must pass was reduced from a width of 0.7 nm to a width of 0.2 nm in an effort to decrease the background absorption.(23) Because this smaller slit width allows less source light to pass through while the electronic noise remains constant, it was anticipated that the signals might display more noise than those measured with a slit width of 0.7 nm. This was proven to be true. The background signal was reduced a bit with the lower 0.2 nm slit width; however, the signals obtained at the 0.7 nm slit width were far more reproducible.

The project continued with the use of a completely different modification solution containing 0.5 mg/L Pd and 2% m/v citric acid in 0.01 mol/L nitric acid, as suggested by Granadillo, Navarro and Romero.(23) In addition, the purge gas was switched from argon to air during the pyrolysis step, in an effort to improve the combustion of the

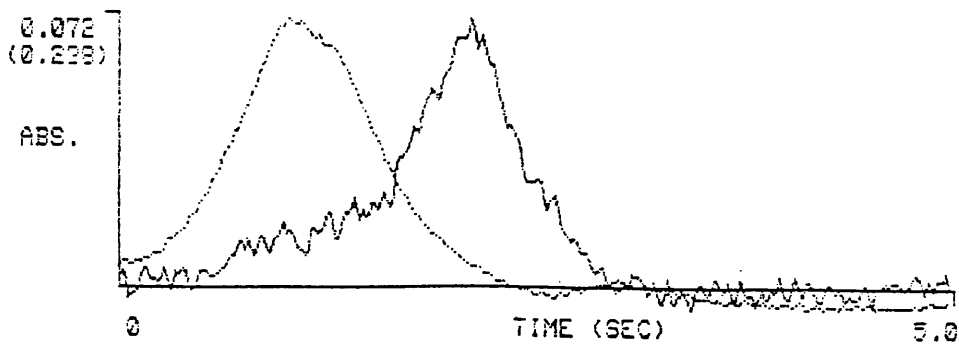
organic matrix. The temperature programs and instrumental parameters prescribed by their work were replicated using a bandpass of 0.7 nm. Precipitation problems were anticipated, so the serum samples were diluted 1:10, 1:4 and 1:1 with 0.1% v/v Triton X-100 and spiked with lead to 5 ppb. The samples and modifiers were dropped onto the platform sequentially instead of being pre-mixed and dropped onto the platform in one shot. For each of these dilution ratios the signal profiles were similar and double peaks were apparent, as seen in Figure 16 for a serum sample diluted 1:9 and modified with the 0.5 mg/L palladium solution. In order to solve this problem, variations in the atomization ramp time were tried for the 1:10 dilutions.



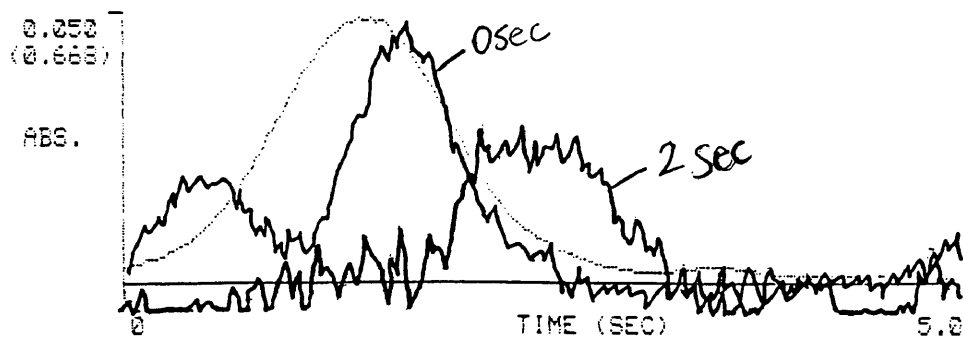
**Figure 16: Absorbance profile for 5 ppb serum diluted 1:10, modified with 0.5 mg/L Pd and 2% citric acid in 0.01 mol/L HNO<sub>3</sub>**

When an atomization ramp of one second was added to the temperature program, the first peak was significantly reduced and the overall peak area was only slightly reduced as seen in Figure 17. The two second atomization ramp produced a single peak for the samples diluted 1:10; however, the profile appeared later in time and the peak area

was significantly reduced. Figure 18 shows a comparison of the peak profiles with no atomization ramp and with a two second ramp. Finally, the three second atomization ramp produced peak profiles for the samples diluted 1:10 that appeared even later than those at the two second ramp; however, the profiles were essentially the same shape. The three second ramp also eliminated the first peak for the samples diluted 1:4; however, the signals were very noisy and not very reproducible.



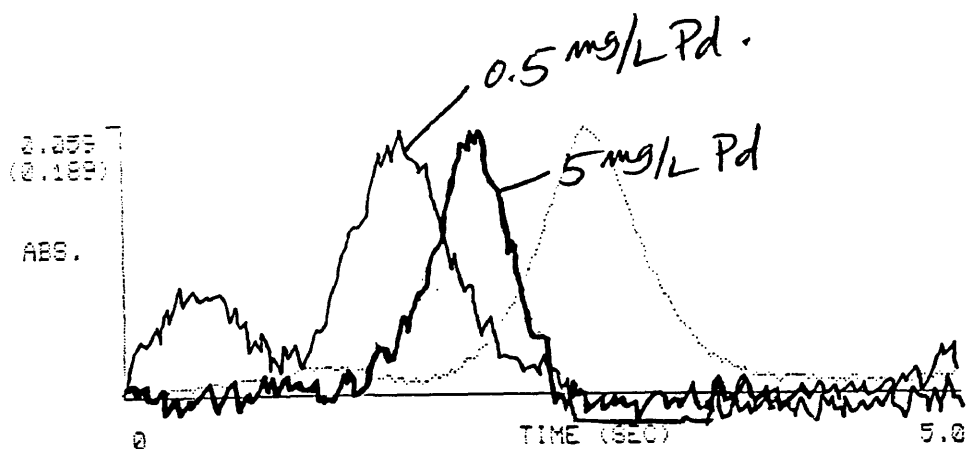
**Figure 17: Profile for serum diluted 1:10, using 1 sec atomization ramp**



**Figure 18: Comparison of profiles using 0 and 2 sec atomization ramp times**

In order to further improve the signal, a greater concentration of palladium was added to the modifier. Higher palladium concentrations are typically used for samples containing high salt concentrations. Earlier attempts to run samples with Pd concentrations in the 0.1-1% range resulted in massive precipitation in the serum samples. Thus, a more gradual increase in the Pd levels was investigated.

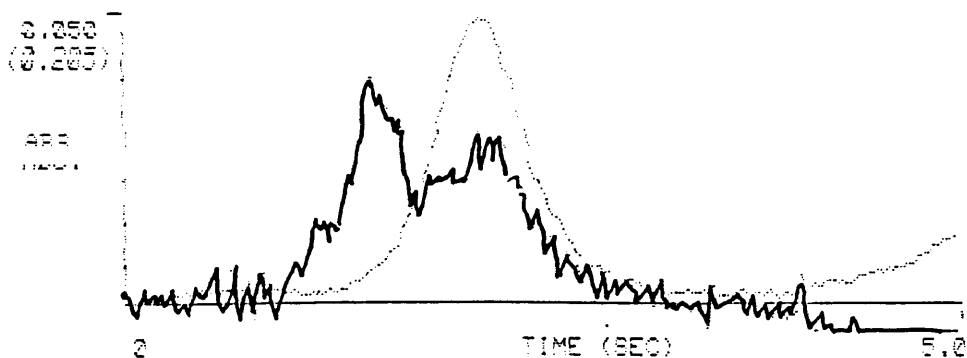
A new modifier solution containing 5 mg/L Pd and 2% m/v citric acid in 0.01 mol/L nitric acid was produced. The resulting lead signals from spiked serum samples were larger and more reproducible, and the first peak disappeared. However, the high background signal was causing the baseline to bottom out. Figure 19 contains a comparison of the profiles from 1:4 dilutions using both palladium concentrations; 0.5 mg/L and 5 mg/L. Higher atomization temperatures of 1800°C and 1900°C were tried in an effort to resolve the problem but they produced no appreciable effects.



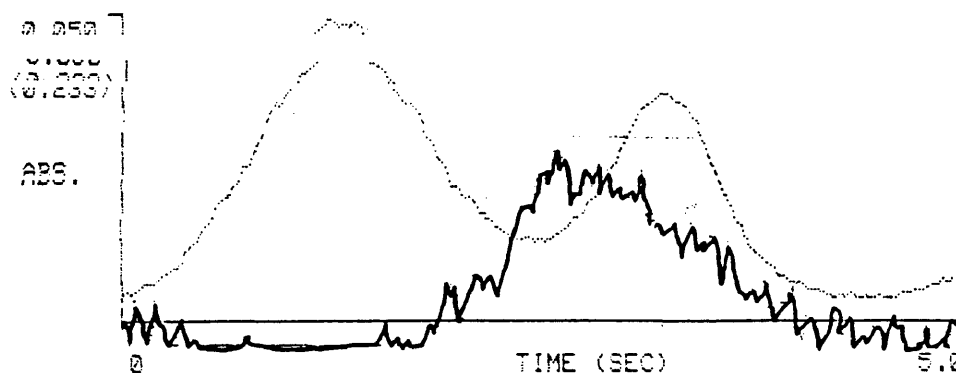
**Figure 19: Comparison of profiles for 1:4 using both modifiers, 0.5 mg/L Pd and 5 mg/L Pd**

As was expected, when the atomization ramp was altered to two seconds with the original atomization temperature of 2000°C the background signal appeared earlier but

further attenuated the signal, as seen in Figure 20. When the atomization ramp was increased to four seconds the background should have appeared later; however, two background peaks were produced which attenuated the signal early on, as seen in Figure 21. There are apparently numerous species present in the matrix which have significant temperature dependencies with respect to the time frame associated with volatilization. Thus, the atomization temperature remained at the recommended 2000°C with a ramp of 3 seconds for further experimentation with modifiers containing higher palladium concentrations.

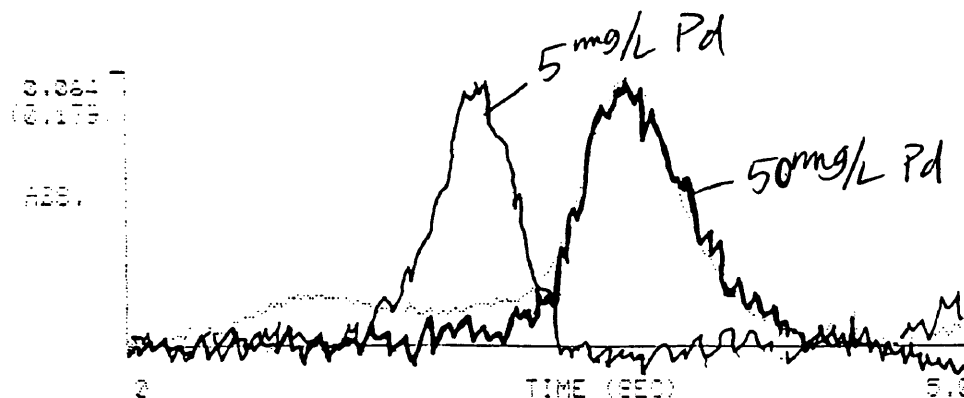


**Figure 20: Peak profile from 2 sec atomization ramp times**



**Figure 21: Peak profile from 4 sec atomization ramp times**

A modifier solution containing 50 mg/L Pd and 2% citric acid in 0.01 mol/L nitric acid was then evaluated. This combination produced signal profiles for the serum samples diluted 1:4 that were much more gaussian in peak shape. A comparison of the signals produced using the modifiers containing 5 mg/L Pd and 50 mg/L Pd is shown in Figure 22. Note that the signal for the 50 mg/L Pd sample appears longer into the atomization time, implying that additional levels of Pd are stabilizing the Pb species with respect to volatilization and atomization temperatures.

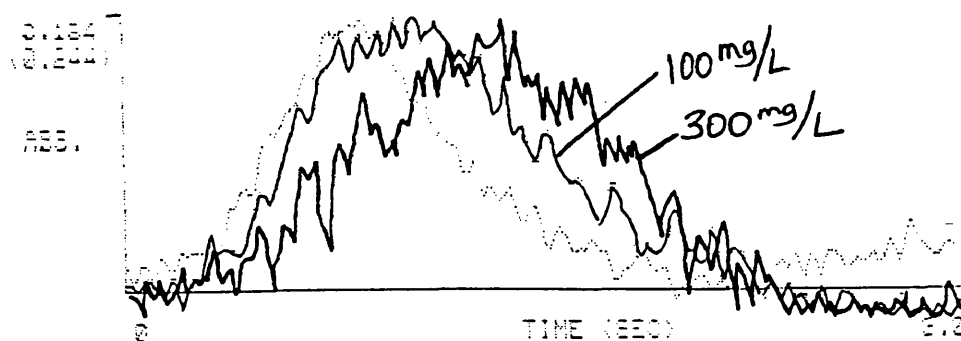


**Figure 22: Comparison of signals produced using the modifiers containing 5 mg/L Pd and 50 mg/L Pd**

In an attempt to further improve the reproducibility and peak profiles, modifier solutions containing the 2% citric acid in 0.01 mol/L nitric acid with various Pd concentrations were evaluated. Modifier solutions were prepared containing 100 mg/L Pd, 200mg/L Pd, 300 mg/L Pd and 500 mg/L Pd. All modifier concentrations were then tested with the furnace conditions prescribed by Granadillo, Navarro and Romero, and with the recommended slit bandpass of 0.2 nm.

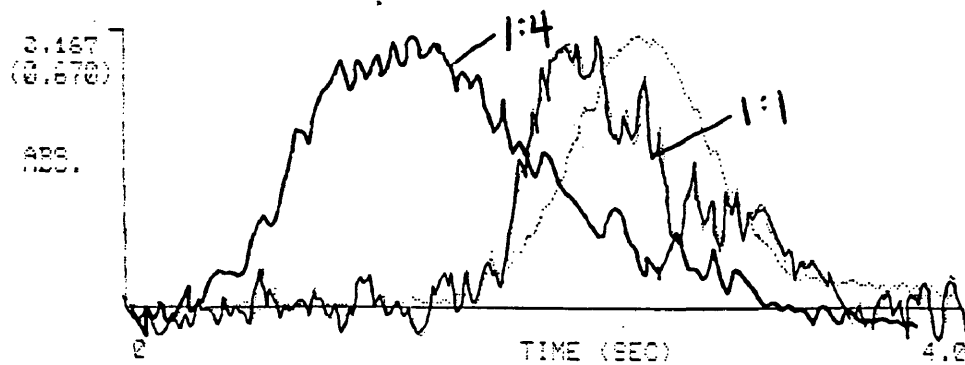
The smaller slit width produced signal profiles that were much more noisy, as expected. As the lead concentrations in the modifier were increased from 50 mg/L to 500 mg/L, some signal was lost and the profiles broadened. Figure 23 shows a comparison of the signals for 1:4 dilutions with 100 and 300 mg/L Pd in the modifier. At the 500 mg/L Pd concentration some background overcompensation was apparent and the signals were prematurely quenched. The signals were most reproducible at the middle of the range of Pd concentrations, in particular from 100 mg/L to 300 mg/L Pd. For the first time in this

project, the signals for samples diluted 1:1 were discernible; however, reproducibility was very poor. In addition, as the dilution ratio was lessened from 1:4 to 1:1 using the same modifier solution, some of the signal was lost and the samples diluted 1:1 appeared later than those diluted 1:4. Figure 24 provides a comparison. It was postulated that species in the matrix were decelerating the atomization of the less dilute samples, possibly due to increased residues on the graphite surface which provided for poorer contact of the lead at the graphite surface during atomization. Thus, the hold time on the char and the atomization temperatures were increased.



**Figure 23: Comparison of profiles for samples diluted 1:4, modifiers with 100 mg/L Pd and with 300 mg/L Pd**

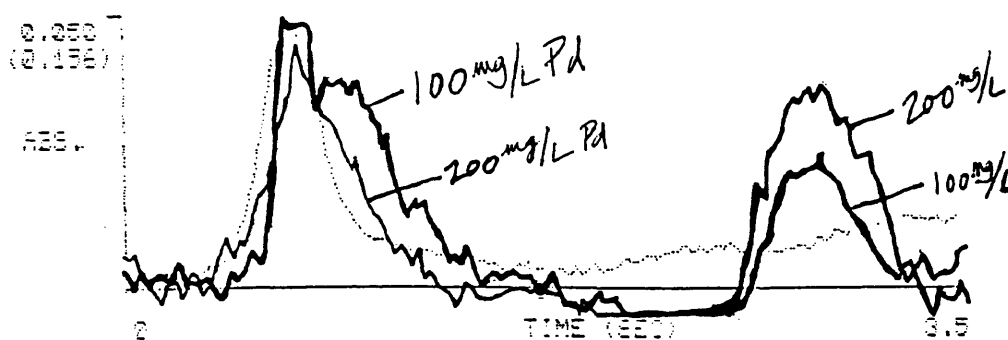




**Figure 24: Comparison of profiles for samples diluted 1:4 and 1:1 using modifier with 100 mg/L Pd**

Continued improvements in the method from this point made use of pig and human plasma instead of serum, which is a matrix closer to whole blood less the cell content. To ensure that this new matrix did not produce any appreciable differences in the signals, the previously tested conditions were used and the signals were essentially unchanged. Next, the char ramp was decreased to 15 seconds and the char hold increased to 30 seconds using the prescribed char temperature of 600°C. Furthermore, the atomization ramp time was increased to 3 seconds, and the hold was increased to 10 seconds at the prescribed temperature of 2000°C. The modifier containing 100 mg/L Pd was used. Unfortunately, the peak profile was significantly changed and double peaks appeared with a quenching apparent between the two. Similar profiles were obtained when the atomization temperature was increased to 2100°C and then to 2200°C. When the modifier containing 200 mg/L Pd was used, the double peaks persisted and the first peak height decreased as the second peak height increased; however, both peak areas

appeared to increase as shown in Figure 25. All variations in atomization temperature produced similar results, and as the char temperature was increased both peak heights decreased. When the Pd concentration in the modifier was increased to 500 mg/L, triple peaks appeared and more peaks may have existed after the integration time cut the signal off.



**Figure 25: Comparison of peak profiles using modifiers with 100 mg/L Pd and 200 mg/L Pd**

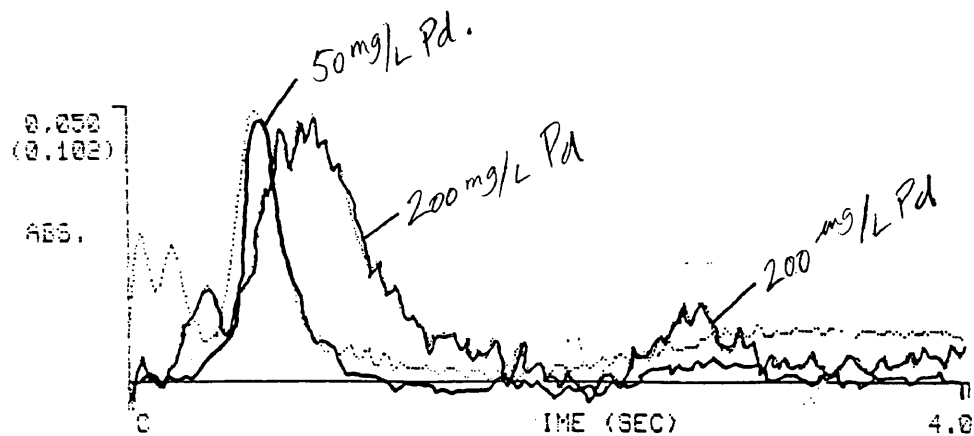
When the originally prescribed conditions were tried again using the modifier containing 100 mg/L Pd, the second peak persisted and the profile was essentially the same. It was postulated that perhaps an absorptive buildup in the tube was causing the additional peaks, and dry fire runs revealed small peaks of residue. Thus, the hold on the cleanout step was increased to five seconds. In addition, runs of just the modifiers containing 100 mg/L Pd and 200 mg/L Pd both revealed numerous peaks, and it was determined that a lead contamination to the modifiers was contributing to the problem. Thus, the graphite tube was changed and new modifiers were produced.

Experimentation resumed with the temperature program using the increased hold times on the char and atomization steps. The temperature program developed to this point is summarized in Table 11. The peak profile for the plasma sample diluted 1:4 using the modifier containing 50 mg/L Pd was clean with only a trace of a second peak. The palladium certainly had an impact on the signals; however, because as the Pd concentration was increased the second peak began to grow and the first peak began to spread out. Figure 26 shows a comparison of the modifiers containing 50 mg/L Pd and 200 mg/L Pd. It was speculated that as the palladium concentration increased, the chance for a matrix-Pd precipitation also increased since Pd significantly enhances precipitation of species in serum and plasma. Thus, the first peak was due to the free Pb, and the second was due to some precipitate that required more time to volatilize in the atomization stage. In an attempt to prevent precipitation, the order in which the modifier and the sample were deposited onto the platform was reversed with the modifier being deposited first. This, however, did not produce an appreciable difference in the signal profiles.

| <b>Step</b>        | <b>Furnace Temperature (°C)</b> | <b>Ramp Time (s)</b> | <b>Hold (s)</b> | <b>Internal Gas Flow (mL/min)</b> |
|--------------------|---------------------------------|----------------------|-----------------|-----------------------------------|
| <b>Dry</b>         | 120                             | 5                    | 5               | 300                               |
| <b>Dry II</b>      | 250                             | 20                   | 10              | 300                               |
| <b>Char</b>        | 600                             | 15                   | 30              | 300*                              |
| <b>Atomization</b> | 2000                            | 0                    | 10              | 0                                 |
| <b>Cleanout</b>    | 2700                            | 1                    | 5               | 300                               |

\*Denotes the alternate gas, air.

**Table 11: Modified temperature program for use with Pd method**

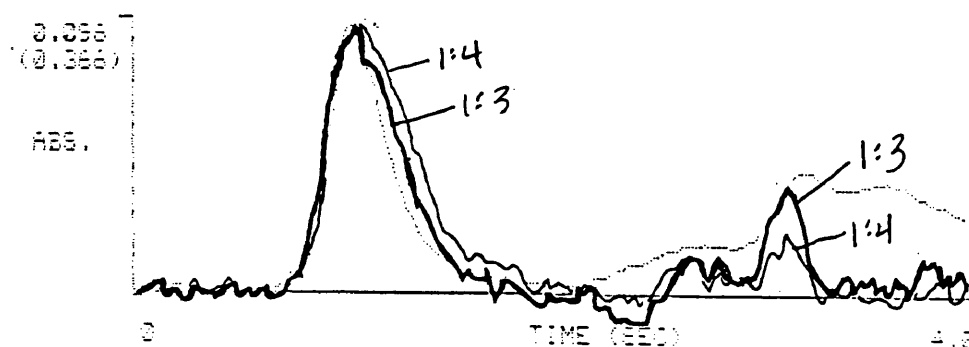


**Figure 26: Comparison of peak profiles using modifiers with 50 mg/L Pd and 200 mg/L Pd**

At this point in time, the GFAAS system was overhauled. This consisted of a thorough cleaning of the furnace, replacement of the contacts and installation of a new graphite tube. When the different modifiers were again compared using the plasma diluted 1:4, the second peak again appeared as the concentration of Pd was increased, and the first peak again broadened. In addition, a slight attenuation of the signal was detected with the growth of the second peak. The modifier containing 200 mg/L Pd appeared to be most reproducible without creating a significant second peak, thus it was used for experimentation throughout the remainder of the project.

As the dilution ratio was lessened from 1:4 to 1:3, the first peak narrowed and the second peak again grew; however, the overall peak height remained relatively constant as seen in Figure 27. As the samples became less dilute, a lead-containing species was being retained longer relative to the majority of the lead, which was more easily volatilized. It was speculated that a small amount of lead might have been imbedded in

the charred material and thus retained, or the two peaks may have been the result of sample splattering during the dry. If the sample was present in two different areas of the tube, two peaks may have resulted due to temperature variations along the length of the tube.

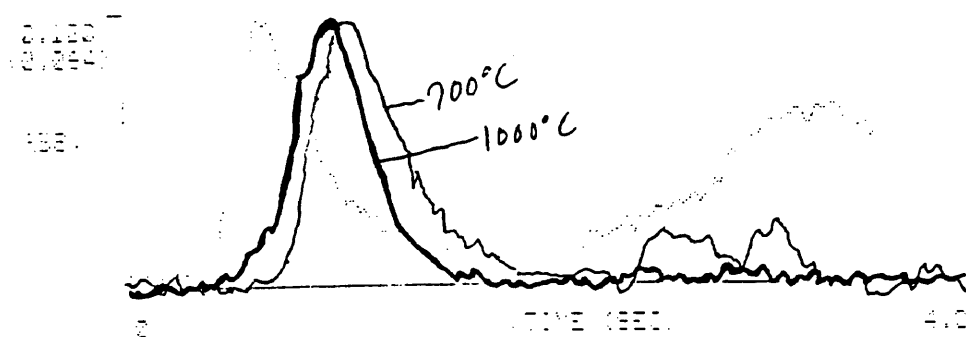


**Figure 27: Comparison of peak profiles for plasma diluted 1:4 and 1:3, modifier containing 200 mg/L Pd**

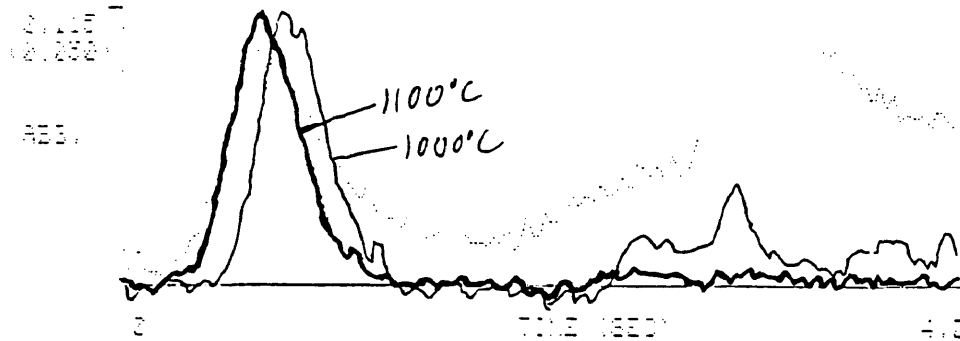
The temperature program that had been used at this point consisted of two dry steps. As the program advanced from one dry to the next, a quiet sizzle was heard. Thus, it was determined that sample splattering did in fact contribute to the double peak problem. In an attempt to reduce possible sample splattering the first dry temperature was increased from 120°C to 150°C with the ramp increased from 5 seconds to 20 seconds and the hold increased from 5 seconds to 20 seconds. The second dry step was eliminated. No splattering was heard with the incorporation of the revised drying temperature program. Sample splattering apparently was not the only contributor to the

double peak problem because the problem worsened with increased Pd concentrations and with lower dilution ratios.

The char temperature was next evaluated from 600°C to 1100°C in 100°C increments. The second peak height significantly decreased until it eventually disappeared as the char temperature was increased. Figure 28 shows a comparison of the profiles for the plasma samples diluted 1:4 using char temperatures of 700°C and 1000°C. The revised drying temperature, used in conjunction with the increased char temperature of 1000°C, produced clean signals with comparable peak heights and areas for samples diluted as low as 1:3. When these conditions were tried using plasma samples diluted at 1:2 the second peak reappeared. However, when the char temperature was further increased to a temperature of 1100°C, the second peak disappeared, the signals were gaussian and reproducible, and the peak areas and heights were comparable to those of the more dilute samples. Figure 29 shows a comparison of the plasma samples diluted 1:2 using the revised dry conditions and char temperatures of 1000°C and 1100°C.



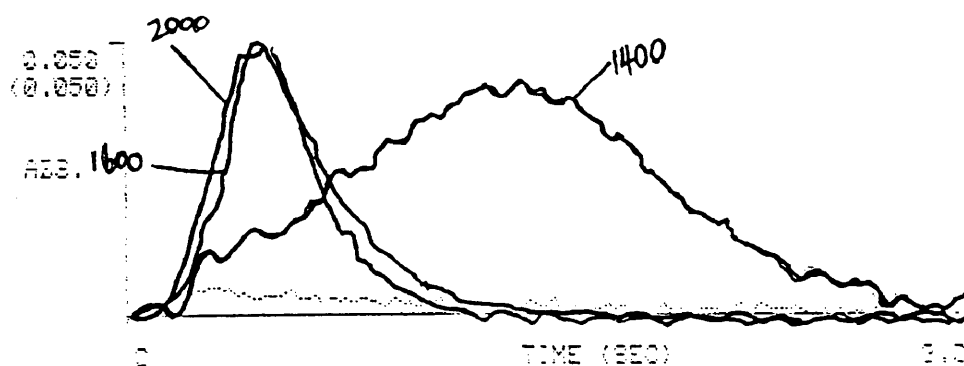
**Figure 28: Comparison of peak profiles for plasma diluted 1:4 using char temperatures of 700°C and 1000°C**



**Figure 29: Comparison of peak profiles for plasma diluted 1:2 using char temperatures of 1000°C and 1100°C**

Next, the char temperature was held at 1100°C as the atomization temperature varied, in an attempt to optimize the atomization temperature. A slit bandpass of 0.7 nm was used. The plasma samples diluted 1:2 were spiked with lead to 30 ppb, in order to increase the signals and facilitate the experimentation. In addition, the signals were electronically magnified by a factor of ten so that additional digits could be evaluated when examining signal reproducibility. As the atomization temperature was decreased from 2000°C, as was originally prescribed by Granadillo, Navarro and Romero, all the way down to an atomization temperature of 1500°C, the peak areas steadily increased, the profiles were essentially unchanged and the reproducibility of the measurements was good. When the atomization temperature was further decreased to 1400°C, the peak areas continued to increase but the peak profile spread out across the entire integration time. Figure 30 shows for a comparison of the peak profiles at various atomization temperatures with the char held constant at 1100°C. At an atomization temperature of

1300°C the signal was significantly decreased, implying that an insufficient temperature was obtained to atomize Pb from the sample.

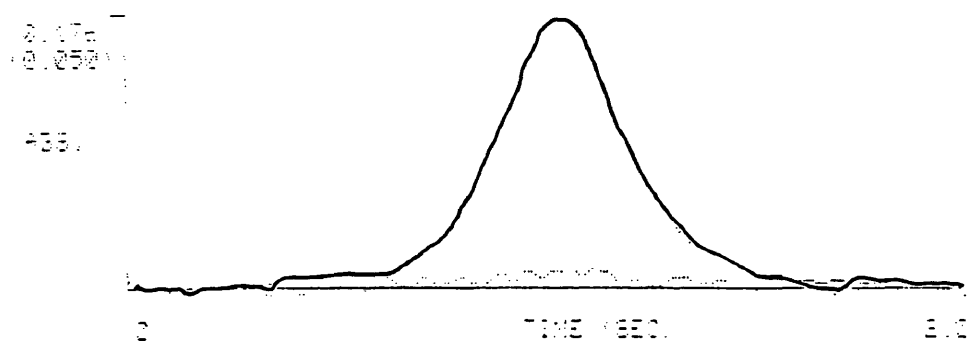


**Figure 30: Comparison of profiles for various atomization temperatures with a char of 1000°C using plasma diluted 1:2**

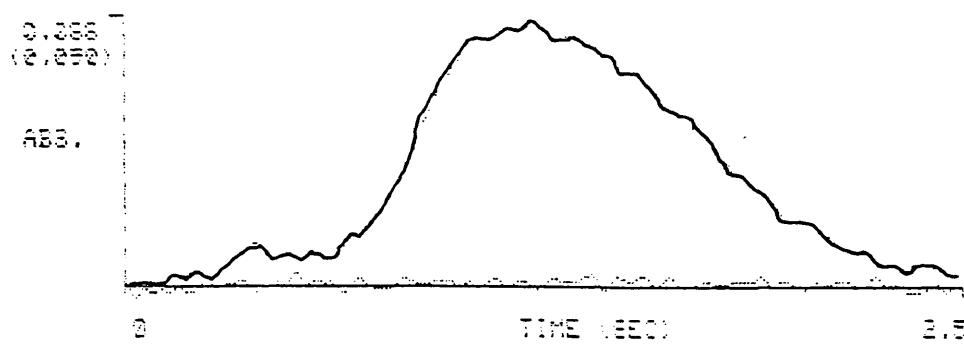
When these temperature changes were tried with aqueous samples spiked to 30 ppb the optimum atomization temperature and the peak profiles were markedly different. The profiles appeared a little later than those of the plasma samples, the peak heights were significantly lower, and the peak areas were lower as well. When the char temperature was held constant at 1000°C the peak areas increased as the atomization temperature was lowered from 2000°C to 1700°C; however, the peaks broadened and the heights decreased. At 1600°C and below the peak areas also began to decrease and a small, early-running peak was apparent. Figures 31 and 32 provide profiles for the aqueous sample at an atomization temperature of 2000°C and 1700°C, respectively. Note that the time scaling on the X-axis is different. A plot of peak area vs. atomization temperature for blood plasma and aqueous standards is given in Figure 33. The points



represent the average peak area for the runs done in triplicate. The relative standard deviations for these points ranged from 0.64% -3.5% for the plasma; however, the relative standard deviation for the point at 1300°C was 20%, as the results were not very reproducible at this atomization temperature. The relative standard deviations for the aqueous samples ranged from 0.33% to 1.6%, except for the atomization temperature of 1400°C. The relative standard deviation at this point was 18.4%, again because the results were not very reproducible at this temperature. An interesting result of this study was that although the optimum atomization temperatures are significantly different for the blood plasma and aqueous standards, the intermediate temperatures of about 1650°C and 1800°C provide an overlap where the net absorbance of both is comparable.



**Figure 31: Profile for a 30 ppb aqueous sample with the char at 1100°C and the atomization at 2000°C**



**Figure 32: Profile for a 30 ppb aqueous sample with the char at 1000°C and the atomization at 1700°C**

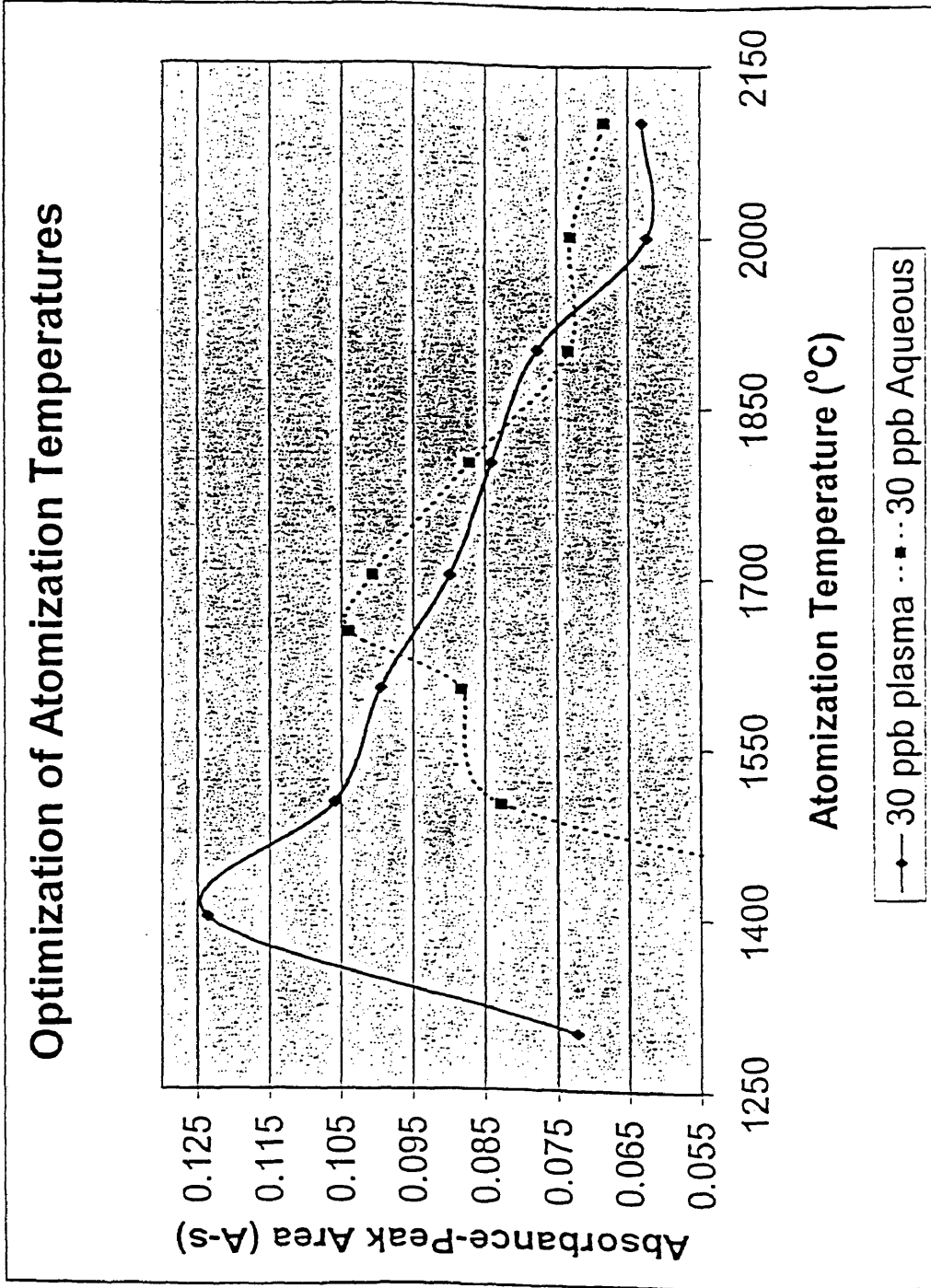
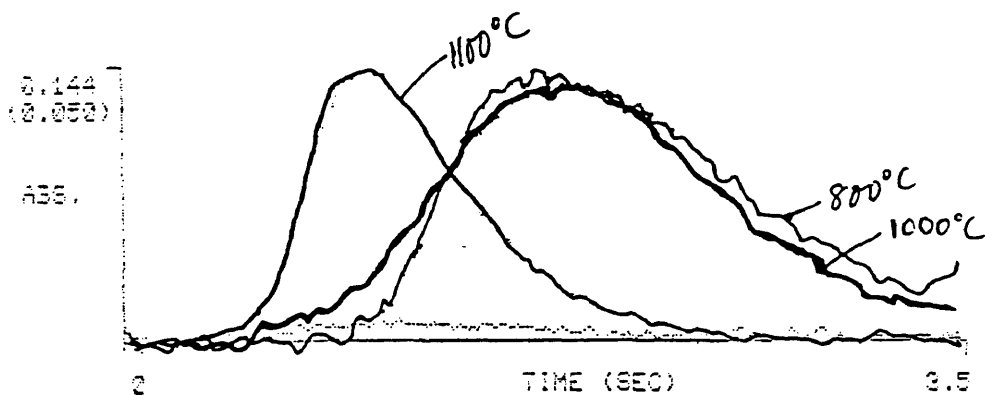


Figure 33: Optimization of atomization temperatures for both aqueous and plasma samples

The char temperature was reevaluated using the plasma samples. The atomization temperature was held constant at 1500°C, as this temperature produced the best results for the plasma. At a char of 1200°C, a clean gaussian peak was obtained, and numerous replicates proved this temperature to be reproducible. As the char temperature was decreased to 1100°C the profile was similar and reproducibility was still good. As the char temperature was lowered even further, stepwise down to 800°C, the peak areas significantly decreased, the background significantly increased, the peak profiles spread out and the peaks appeared later with lower reproducibility. Figure 34 shows a comparison of these profiles and Figure 35 a graphical interpretation of these results. The points represent the average peak area for runs taken in triplicate, and the relative standard deviations for these points ranged from 0.23% to 4.6%.



**Figure 34: Comparison of profiles for various char temperatures with an atomization of 1500°C using plasma diluted 1:2**

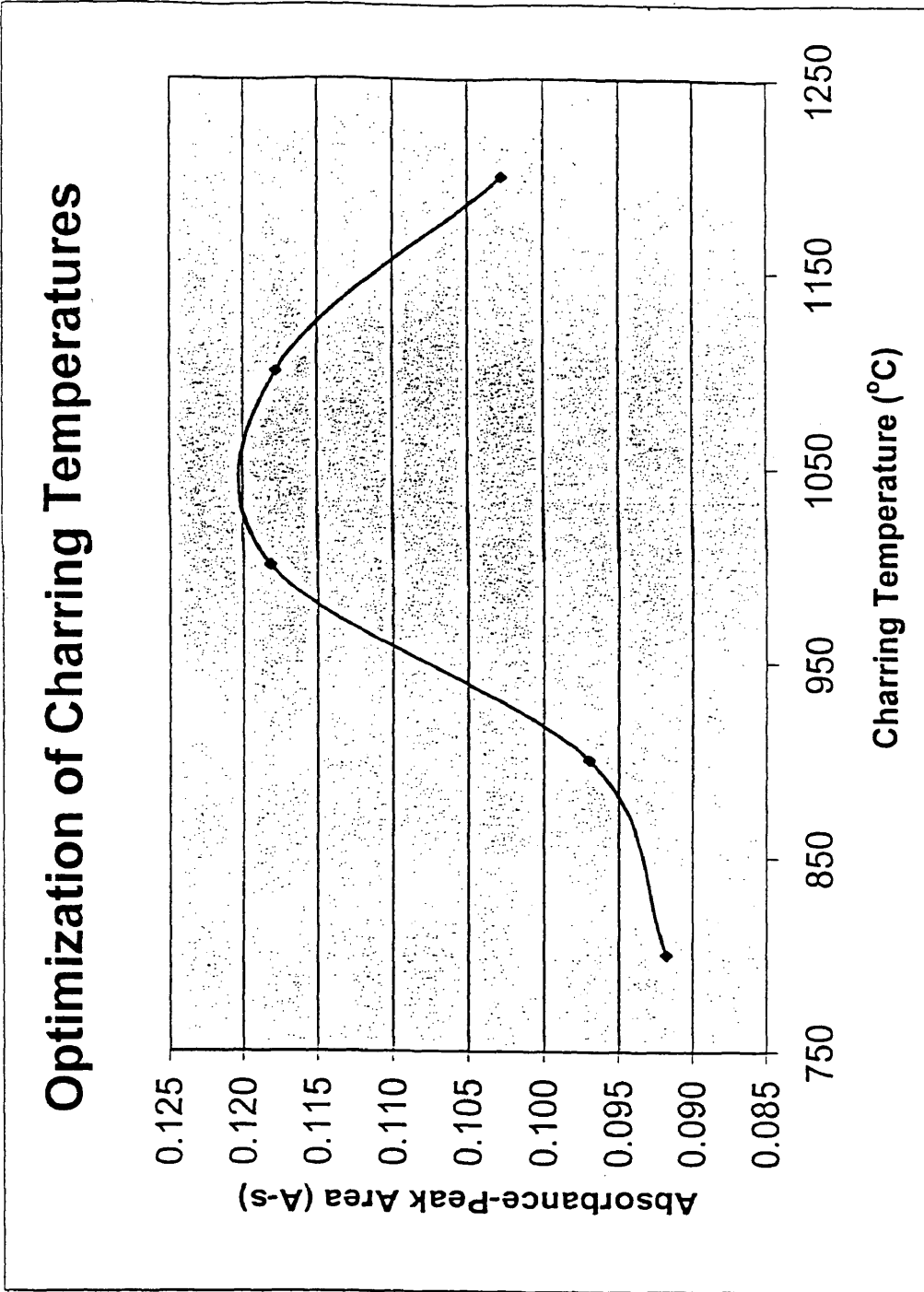


Figure 35: Optimization of charring temperatures

An examination of Figure 33 indicated that at the optimum charring temperature of 1000°C, the peak areas for the plasma samples and aqueous samples should be comparable if an atomization temperature of about 1650°C, 1800°C or 1900°C was used. Of these temperatures, 1650°C was closest to the optimal atomization temperature for blood plasma (about 1400°C-1500°C). Thus, at an atomization of 1650°C a calibration curve could theoretically be generated from aqueous standards which would match signals generated from the blood plasma.

The optimized procedural conditions used up to this point are summarized in Table 12. A calibration curve was generated from aqueous standards spiked with lead to give concentrations of 5, 15, 30 and 50 ppb. Plasma samples were diluted 1:2 and also spiked to the same concentrations. Figure 36 provides a comparison of the peak profiles for both matrices spiked to 30 ppb. The matrix certainly has an effect, as the profiles are markedly different. The plasma peak appears first, and the aqueous peak is broader with a lower peak height; however, the overall peak areas are essentially the same. Calibration curves generated from both matrices are provided in Figure 37. Slopes were generated from a linear regression fit using Excel. The slope for the aqueous standards was .0021, and that for the plasma samples was .0022. The similarities imply that the matrix effects are minimal if peak areas, instead of peak heights, are considered.

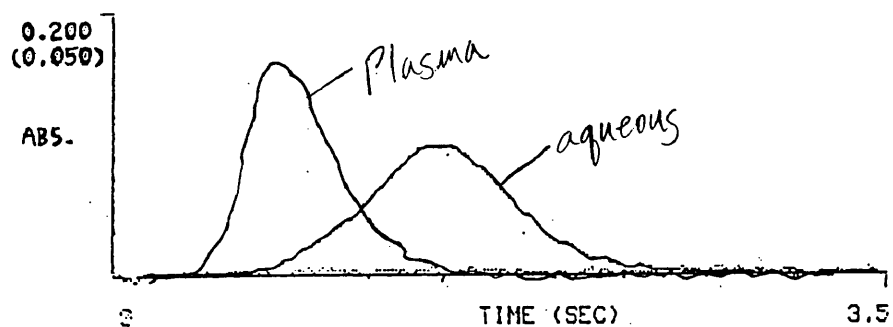
| Step        | Furnace Temperature (°C) | Ramp Time (s) | Hold (s) | Internal Gas Flow (mL/min) |
|-------------|--------------------------|---------------|----------|----------------------------|
| Dry         | 150                      | 20            | 20       | 300                        |
| Char        | 1000                     | 15            | 30       | 300*                       |
| Atomization | 1650                     | 0             | 5        | 0                          |
| Cleanout    | 2700                     | 1             | 5        | 300                        |

\*Alternate gas, air

Modifier Solution: 200 mg/L Pd and  
2% w/v Citric Acid in  
0.01 mol/L Nitric Acid

Plasma Sample Dilution: 1:2 with 0.1% v/v Triton X-100

**Table 12: Optimized procedural conditions for plasma**



**Figure 36: Comparison of peak profiles for both matrices spiked to 30 ppb**

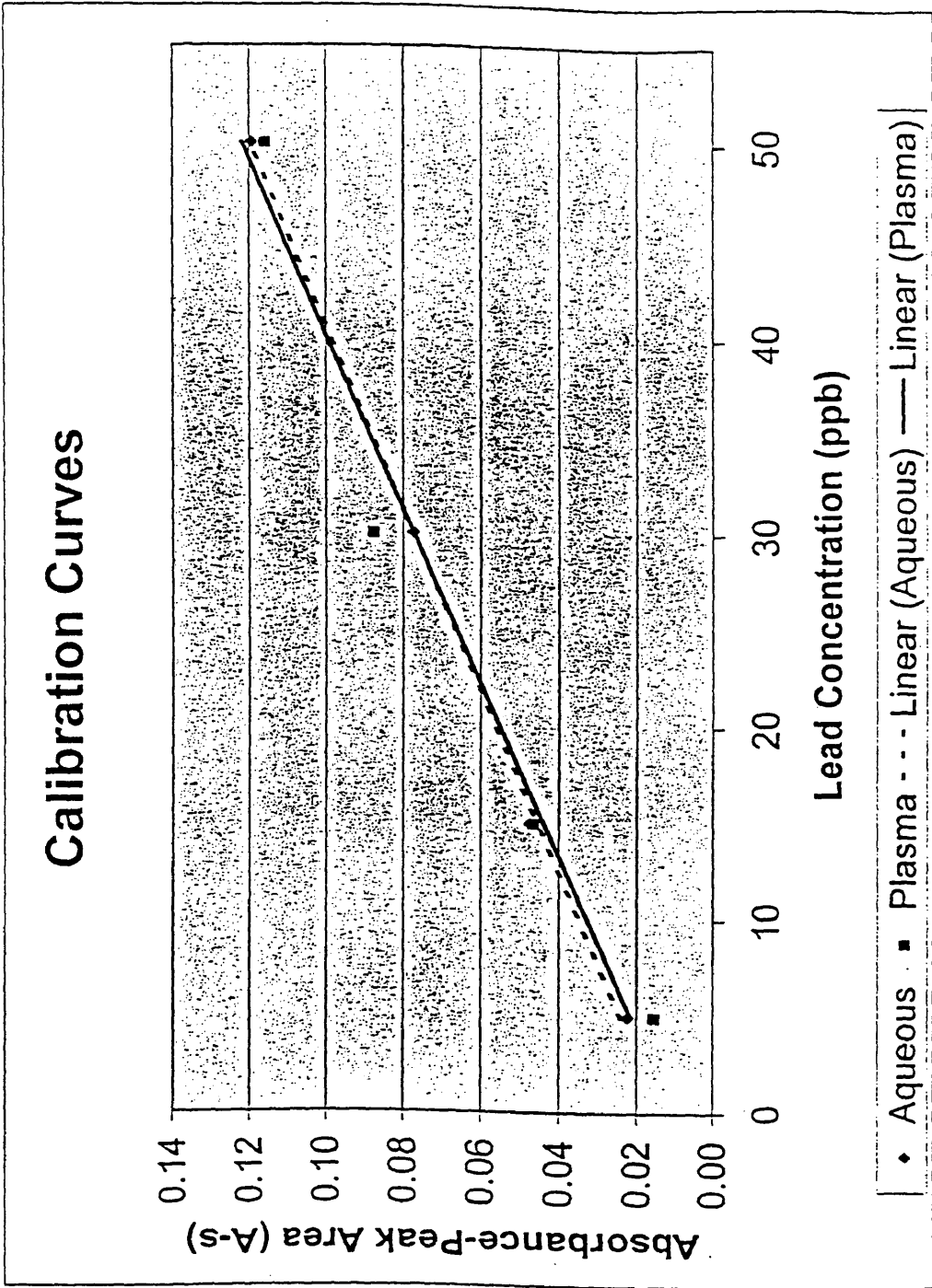
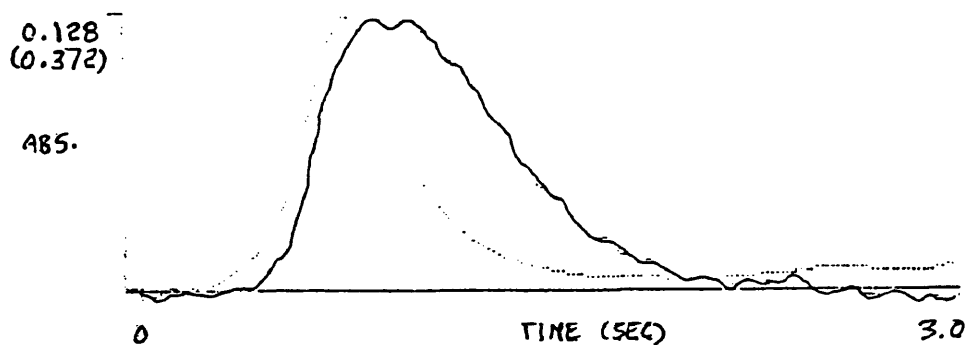


Figure 37: Calibration curves for plasma

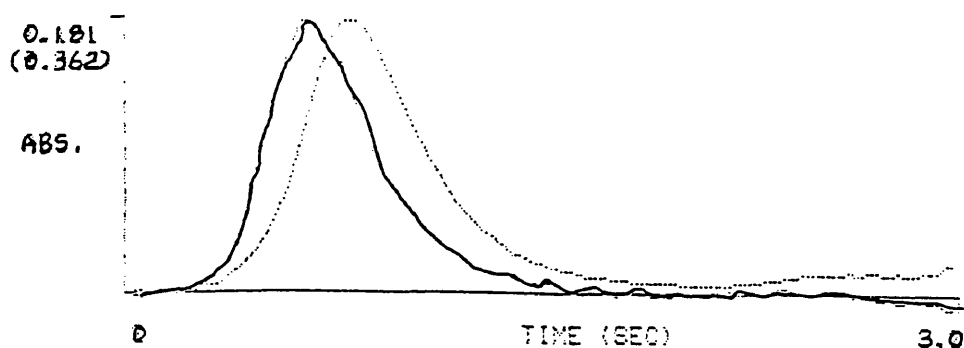


Next, a whole blood sample was diluted 1:2 with 0.1% v/v Triton X-100, spiked with lead to 30 ppb and run under the same instrumental parameters. Initially, the blood samples did not drop directly onto the L'vov platform but instead climbed up the sides of the graphite tube due to high surface tension. Thus, blood samples were diluted with a greater concentration of Triton X-100 surfactant (0.5% v/v). This solved the problem and peak profiles were obtained; however, the peak areas were only about 60% of the expected value. Thus, the char temperature was increased from 1000°C to 1100°C in an effort to increase the signal; however, all signal was lost. Next, a char temperature of 1050°C was used and only a hint of a peak was apparent. When the char temperature was lowered to 900°C, the peak areas were equal to those of aqueous standards run with the same temperature program.

Using the 900°C char temperature, the peak profiles for the blood samples were sharper than those of the plasma samples, and they also appeared earlier in time. In addition, the plasma samples gave absorbance peak areas that were erroneously high when compared to the signals for the aqueous standards. Figure 38 shows a peak profiles for plasma, and Figure 39 shows one for blood. This comparison indicates that the organic load has a substantial impact on the lead volatility and atomization rate.



**Figure 38: Peak profile for 30 ppb plasma sample diluted 1:2**



**Figure 39: Peak profile for 30 ppb whole blood sample diluted 1:2**

Analytical signals from the blood samples were very reproducible. For five runs of the sample spiked with lead to 30 ppb, an average peak area of 0.0846 A-s was obtained with a relative standard deviation of 4.3%. An unspiked blood sample which was run five times gave an average peak area of 0.0098 A-s with a relative standard deviation of 0.92%. Based on Beer's law, if a signal for 30 ppb gave a reproducible

average peak area of 0.0846 A-s; a reproducible average peak area of 0.0098 would represent a signal of about 3.4 ppb. As the unspiked blood samples gave very clean and reproducible peak profiles with an average peak area of 0.0098, it can be estimated that the limit of detection associated with this method is in the 0.5-1 ppb range. The final temperature program and matrix modifiers used to obtain these results are provided in Table 13.

| <b>Step</b>        | <b>Furnace Temperature (°C)</b> | <b>Ramp Time (s)</b> | <b>Hold (s)</b> | <b>Internal Gas Flow (mL/min)</b> |
|--------------------|---------------------------------|----------------------|-----------------|-----------------------------------|
| <b>Dry</b>         | 150                             | 20                   | 20              | 300                               |
| <b>Char</b>        | 900                             | 15                   | 30              | 300*                              |
| <b>Atomization</b> | 1650                            | 0                    | 5               | 0                                 |
| <b>Cleanout</b>    | 2700                            | 1                    | 7               | 300                               |

\*Alternate gas, air

Modifier Solution: 200 mg/L Pd and  
2% w/v Citric Acid in  
0.01 mol/L Nitric Acid

Plasma Sample Dilution: 1:2 with 0.5% v/v Triton X-100

**Table 13: Optimized procedural conditions for blood**

A calibration curve was generated from aqueous standards spiked with lead to give concentrations of 5,15,30 and 50 ppb. Blood samples were diluted 1:2 with the 0.5% v/v Triton X-100 and also spiked to the same concentrations. The resulting curves are provided in Figure 40. Slopes were again generated from a linear regression fit using Excel. The slope for the aqueous standards was 0.0018, and that for the blood samples

was 0.0010. Although the aqueous curve is a little higher than the blood curve, the similarities again imply that the matrix effects are insignificant if peak area, instead of peak heights, are considered. Time did not allow for further refinements in the method.

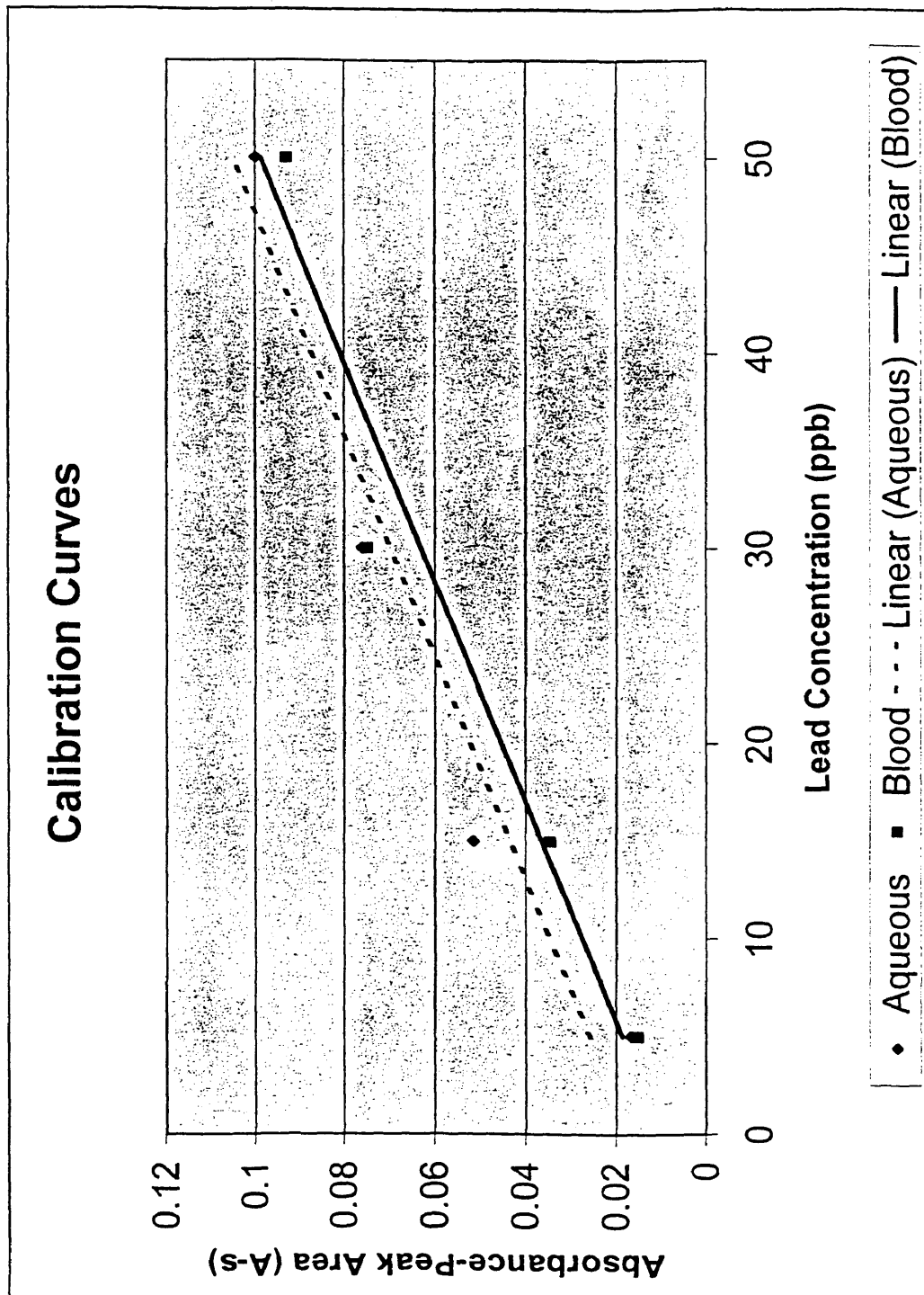


Figure 40: Calibration curves for whole blood

An explanation for this difference in the peak profiles can be derived from an explanation of the atomization mechanism postulated by Granadillo, Navarro and Romero.(23) The mechanisms by which palladium actually interacts with the lead analyte are still being debated. However, it is apparent that any model intended to elucidate the atomization mechanism must consider the following circumstances: (1) the type of chemical and/or physical interactions existing between the graphite surface, the analytical matrix modifier and the analyte; (2) the reductive capability of the carbon at the high operating temperatures of the ETAAS technique; and (3) the availability of active carbons within the graphite furnace to reduce the atomic precursor to the analyte atoms. Thus, Granadillo, Navarro and Romero considered the effects that increasing amounts of potentially reducing carbon, produced by the addition of carbon-containing compounds to the samples, exerted on the absorbance-time profiles of lead atomized in the graphite furnace. In addition, the reductive action of the carbon in the graphite furnace itself was considered.

Several researchers have established that the graphite surface plays a major role in the atomization of any metal in GFAAS.(12,16,17) The graphite surface consists of crystallites of graphite with carbon atoms lying in a basal plane which is terminated by carbons in zigzag and armchair configurations.(23) These edge carbons constitute the active surface area of the graphite because they have unpaired  $\sigma$ -electrons available to form bonds, whereas the basal plane carbons have their  $\sigma$ -electrons tied up in bonds with the adjacent carbons.

Previous work has indicated that the use of reducing agents to promote the reduction of palladium is essential to obtain the modification effect.(23) In this study, the citric acid was utilized for this purpose to create a better reducing environment by providing active carbon sites different from those found on the graphite furnace. Thus, in the presence of the citric acid, the carbon sites of the furnace were less susceptible to oxidation and the lifetime of the graphite structure was prolonged.

When no citric acid was added to either the aqueous or blood samples they discovered that the fastest lead atomization occurred in the blood samples due to the high organic content.(23) Apparently, an additional supply of reducing carbons, different from those provided by the graphite furnace, were available from the carbonaceous matrix. However, when 200 µg of citric acid was added to both the aqueous and blood samples, the lead profiles appeared at the same early time. Furthermore, this phenomenon occurred with the exact same profile appearance time for both the palladium modification solution and also for a phosphate-magnesium modification solution.

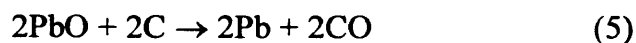
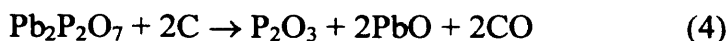
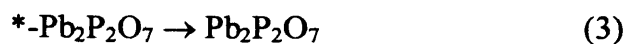
These results suggested that the atomization rate of lead was independent of the matrix and of the modifier used, and that a carbon-dependent mechanism occurred.(23) The differences in the plasma and blood peak profiles provided in Figures 38 and 39 added further credence to this theory. The blood samples, which contained a higher carbonaceous load, appeared much earlier than the plasma samples.

Granadillo, Navarro and Romero proposed the following mechanisms for the atomization of lead by GFAAS with palladium modification [equations (1) and (2)] and

with magnesium-phosphate modification [equations (3) through (5)], assuming that a carbon source other than the graphite surface was available.(23)



and



\* denotes carbon surface of graphite tube

The intermediate species, such as PbO and  $\text{Pb}_2\text{P}_2\text{O}_7$ , were not identified in this study due to a lack of instrumental facilities; however, further experimentation added credence to the postulated atomization mechanism.(23) The redox reaction between  $\text{Pb}_2\text{P}_2\text{O}_7$  and 2 mol of carbon atoms, as postulated, would result in the formation of 1 mol of  $\text{P}_2\text{O}_3$ , 2 mol of PbO and 2 mol of CO. A further 2 mol of carbon would then be required for the reduction of the 2 mol of PbO to form 2 mol of Pb atoms and 2 mol of CO. Therefore, 4 mol of carbon atoms would be needed for the over-all reaction if it occurred as written. This was verified experimentally when four times more mass of



citric acid was needed to reduce the phosphate-magnesium modification than for the palladium modification technique, with the same appearance time for the analyses of the samples and aqueous standards.

Additional support for the proposed mechanism was obtained when atomization was attempted under a partial pressure of CO, and it was discovered that the absorbance profiles for both standards and samples appeared later in time.(23) It was postulated that the time shift in the appearance of the absorption pulse occurred as a result of a suppression of the redox reaction that produced atomic lead, as described by equation (2), in response to the increase in the partial pressure of CO. The carbon monoxide atmosphere caused the system at equilibrium to shift to the reactant side.

In conclusion, the proposed carbon-induced reduction of the atomic precursor may be the mechanism to occur for any analyte that undergoes atomization via the oxide.(23) However experimental verification, such as by GFAAS-mass spectrometry, of the existence of the postulated intermediates in the graphite furnace is required to further substantiate this atomization mechanism of lead.

## **Chapter VI: Summary**

The use of GFAAS for the determination of lead in blood has increased significantly in the past decade.(18) Most of the published methods utilize the Zeeman background correction system; however, this system is not readily available in many testing laboratories and the methodology utilized was found not to be effective with a more conventional deuterium arc background correction system. This led to the development of refined matrix modification and atomization procedures which gave a good reproducibility and sensitivity, and also a good linear range of determination for lead in blood. The limits of detection in particular were significantly improved by a factor of about ten over values reported in the literature by reducing the dilution ratio necessary for the blood analysis.

Work with whole blood samples was only performed over the very last stages of this project, thus there are several parameters that need to be addressed and refined. In particular, an optimization of the furnace and temperature conditions such that all signals are equivalent regardless of whether the samples are aqueous, plasma or whole blood would be very beneficial. In addition, further verification of the reproducibility needs to be addressed in particular with the whole blood samples at low concentrations (<10 ppb).

Finally, the complete linear working range for lead concentrations in blood must be determined.

Reproducibility will be a crucial consideration based on the CDC requirement that clinical decisions be made for children whose blood Pb level is above 150 ppb. For instance, suppose a physician is monitoring a child whose true BPb is at a dangerous level of 170 ppb. The blood analyses at two laboratories both work with reproducibilities within an accepted + or – 20 ppb limit. The physician may face a situation where one laboratory returns a result of 150 ppb, which may not require intervention, and the other returns a 190 ppb result, which would require intervention. Clearly, the burden on public health officials would be lessened if laboratories could operate within a +/- 1-5 ppb limit at low levels. The method described by this work appears to have good reproducibility as referenced by the relative standard deviation of 4.3% for 30 ppb blood samples. This standard deviation is clearly reproducible enough to give good clinical reliability; however, continued refinements to the methodology may be necessary for accurate quantification and use in large scale screening programs.

## References

- (1) Hammond, P.B.: 1982. *Inorganic Lead in Man's Environment: Sources and Toxicological Significance*, J. of Applied Toxicology, 2(2), pp. 68-74.
- (2) Lippman, Morton: 1990. *Lead and Human Health: Background and Recent Findings*. Environmental Research, 51, pp. 1-24.
- (3) Needleman, Herbert L. Human Lead Exposure. Boca Raton, Florida: CRC Press Inc., 1992.
- (4) Angle, C.R., et. at.: 1975. *Lead: Environmental Sources and Red Cell Toxicity in Urban Children*. Report, U.S. Environmental Protection Agency, PB-249061. Gov. Rep. Announce. Index, 76(7), pp. 89.
- (5) Elwood, Peter C.: 1986. *The sources of Lead in Blood: A Critical Review*. The Science of the Total Environment, 52, pp. 1-23.
- (6) Underwood, Eric J.: 1979. *Environmental Sources of Heavy Metals and their Toxicity to Man and Animals*. Progress in Water Technology, 11(4,5), pp. 33-45.
- (7) Submaranian, Kunnath S. and Jean C. Meranger: 1981. *A Rapid Electrothermal Atomic Absorption Spectrophotometric Method for Cadmium and Lead in Human Whole Blood*, Clinical Chemistry, 27(11), pp. 1866-1871.

- (8) Goyer, Robert A.: 1996. *Results of Lead Research: Prenatal Exposure and Neurological Consequences*. Environmental Health Perspectives, **104**(10), pp. 138-140.
- (9) Blaha, K., Bencko, V. and M. Cikrt: 1996. *Exposure to Lead and Human Health in the Czech Republic*. Central European J. of Public Health, **4**, pp. 233-241.
- (10) Parsons, Patrick J. and Walter Slavin: 1993. *A Rapid Zeeman Graphite Furnace Atomic Absorption Spectrometric Method for the Determination of Lead in Blood*, Spectrochimica Acta, **48B**(6,7), pp. 925-939.
- (11) Baxter, Douglas C. and Wolfgang Frech: 1995. *Speciation of Lead in Environmental and Biological Samples*, Pure and Applied Chemistry, **4**, pp. 615-648.
- (12) The Perkin-Elmer Corporation, Techniques in Graphite Furnace Atomic Absorption Spectrophotometry. 1985.
- (13) Skoog, D.A. Principles of Instrumental Analysis. New York, New York: Saunders College Publishing, 1985.
- (14) Wassell, M.J. Direct Determination of Trace Metals in Saline Water Samples using GFAAS, The College of William and Mary Publishing, 1994.
- (15) Pyrzynska, Krystyna: 1996. *Organolead Speciation in Environmental Samples: a Review*, Mikrochimica Acta, **122**, pp. 279-293.
- (16) The Perkin-Elmer Corporation, Analytical Methods for Atomic Absorption Spectrophotometry. 1982.
- (17) Beaty, R.D., Concepts, Instrumentation and Techniques in Atomic Absorption Spectroscopy. The Perkin-Elmer Corporation, 1988.

- (18) Eaton, David K. and James A. Holcombe: 1993. *Oxygen Ashing and Matrix Modifiers in Graphite Furnace Atomic Absorption Spectrometric Determination of Lead in Whole Blood*, Analytical Chemistry, **55**, pp. 946-950.
- (19) Fernandez, Frank J: 1975. *Micromethod for Lead Determination in Whole Blood by Atomic Absorption, with Use of the Graphite Furnace*, Clinical Chemistry, **21**(4), pp. 558-561.
- (20) Fernandez, F.J. and D. Hilligoss: 1982. *An Improved Graphite Furnace Method for the Determination of Lead in Blood Using Matrix Modification and the L'vov Platform*, Atomic Spectroscopy, **3**(4), pp. 130-131.
- (21) Pruszkowska, E., et. al.: 1983. *Blood Lead Determination with the Platform Furnace Technique*, Atomic Spectroscopy, **4**(2), pp. 59-61.
- (22) Hodges, Denis J. and Douglas Skelding: 1983. *Determination of Lead in Blood by Atomic-Absorption Spectroscopy with Electrothermal Atomization*. Analyst, **108**, pp. 813-820.
- (23) Granadillo, Victor A., et. al.: 1993. *Graphite Furnace Atomization Behaviour of Lead Contained in Clinical and Environmental Materials in the Presence of Palladium-induced Isoformation and Citric Acid*, J. of Analytical Atomic Spectrometry, **8**, pp. 615-622.

## VITA

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