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Determination of Lead in Whole Blood by Filter Furnace Laser-Excited Atomic Fluorescence Spectrometry

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Filter furnace laser-excited atomic fluorescence spectroscopy was used for the determination of lead in whole blood. The use of a Katskov filter has enabled the direct comparison between lead fluorescence signals obtained with blood standards and water standards. Only a simple dilution of the blood was required. This direct correlation has allowed the interpolation of the lead signal of blood samples using a lead water standard calibration curve. NIST certified blood samples were analyzed using the method with excellent results.

Keywords Filter furnace laser-excited atomic fluorescence spectrometry, blood, lead, calibration curve method

Electrothermal atomization is considered one of the most efficient means to atomize samples prior to the detection of atoms by either fluorescence or absorption. However, this technique is far from perfect because of the interactions that can take place among the analyte, the carbon of the graphite atomizer and principally the components from the matrix containing the analyte of interest. Analytical matrices containing high amounts of organic matter very often present problems related to the decrease of analyte sensitivity. The two main causes are losses of analyte during the heating cycle because of formation of volatile molecular containing the analyte of interest and/or increases of background of molecular origin. The problem is usually addressed and, in many cases, minimized by the optimization of the temperature program together with stabilized temperature platform furnace (SPTF) technique[1,2] and by chemical modification.[3]

Although the choice of proper ashing temperatures and heating rates is very effective for elements with high thermal and chemical stability, this approach does not seem to be always effective for elements which form volatile molecular species like lead.[4] A platform, which delays the atomization of the analyte until a temperature equilibrium is reached between the walls and the center of the tube, in many cases is a very useful approach to minimize problems related with the formation of compounds after the vaporization of highly volatile and medium volatile elements. Chemical modifiers can chemically transform the analyte of interest making it less volatile, and so higher ashing and/or atomization temperatures can be applied. Chemical modifiers also can be employed to interact with interfering species in order to achieve a time-resolved release of products during the heating cycle achieving a separation of the analyte from the matrix components. A disadvantage of using chemical modifiers is that they are usually employed in concentrations of the order of thousands of times higher than the analyte concentration present in the sample. This procedure can introduce impurities into the sample and cause false analytical results, principally if detection of ultratrace amounts of a common element like lead is intended. In addition, finding an appropriate chemical modifier is not always guaranteed.

The graphite filter furnace (FF) is a new concept of electrothermal atomization, recently introduced by Katskov[5] and it has been applied successfully in atomic absorption spectroscopy.[6] The possibility of the introduction of larger volumes of sample, the decreased pre-atomization time and temperatures, and the elimination of spectral background as well as chemical interferences without the use of chemical modifiers are improvements that the FF technique have to offer over the commonly used atomization techniques. The spool shaped graphite filter, made of porous graphite, is inserted inside a pyrolytic graphite tube where the sample is deposited in the gap between the wall of the tube and the filter, outside the analytical zone. During the heating process, the matrix vapor is diffusionally delayed by the porous graphite and physically separated from the analytical zone, resulting in a temporal resolution between atomic and molecular background signals.

In this work, the filter furnace atomization is employed for direct analysis of lead in blood by laser-excited atomic fluorescence. Lead is an element with relatively high volatility, which complicates the use of pre-atomization steps without loss of analyte in complex matrices. Molecular background, even though it is much less a problem for atomic fluorescence than for atomic absorption, can degrade limits of detection in the analysis of samples with high content of organic

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matter. The interference caused by matrix components can decrease the analytical signal of the analyte compared to aqueous analyte standards of the same concentration. Ideally, a method to analyze complex samples should allow the correlation of complex standards to aqueous standards simplifying the calibration curve procedure by eliminating the need for blood standards. In previous work by Wagner *et al.*[4], correlation was successfully accomplished for lead by using a photodiode placed behind the furnace, in line with the laser beam, correcting for the decrease of laser intensity of the sample relative to the blank. Potentially, the Katskov filter is a cheaper and simpler alternative to achieve such correlation of standards. In this paper, the filter furnace technique is employed as the atomization technique to achieve correlation of the lead fluorescence intensity between aqueous and blood standards, simplifying the determination procedure.

Experimental

Instrumentation

The instrumentation employed in this work is shown in Fig. 1 and it is described in details elsewere.[7,8] Briefly, a dye laser (Model DLII, Molectron) pumped by a copper vapor laser (Model CU15-A, Oxford Lasers, Acton, MA) at repetition rate of 10 kHz was employed as the excitation source. Rhodamine 590 (Exciton Corp., Dayton, OH) cooled in a refrigerator unit was constantly pumped through the oscillator and amplifier dye laser cells. The laser output was frequency doubled using a KDP crystal in order to obtain the 280.30 nm line used to excite the Pb atoms. Typical energy output obtained after frequency doubling was 2 □J with a pulse duration of 4 ns. A graphite furnace (Perkin Elmer, Model HGA-400, Norwalk, CT) modified with silica windows mounted at 45° was used to atomize the samples. An electronic bypass (solenoid valve) (NR research Inc., Caldwell NJ) was employed

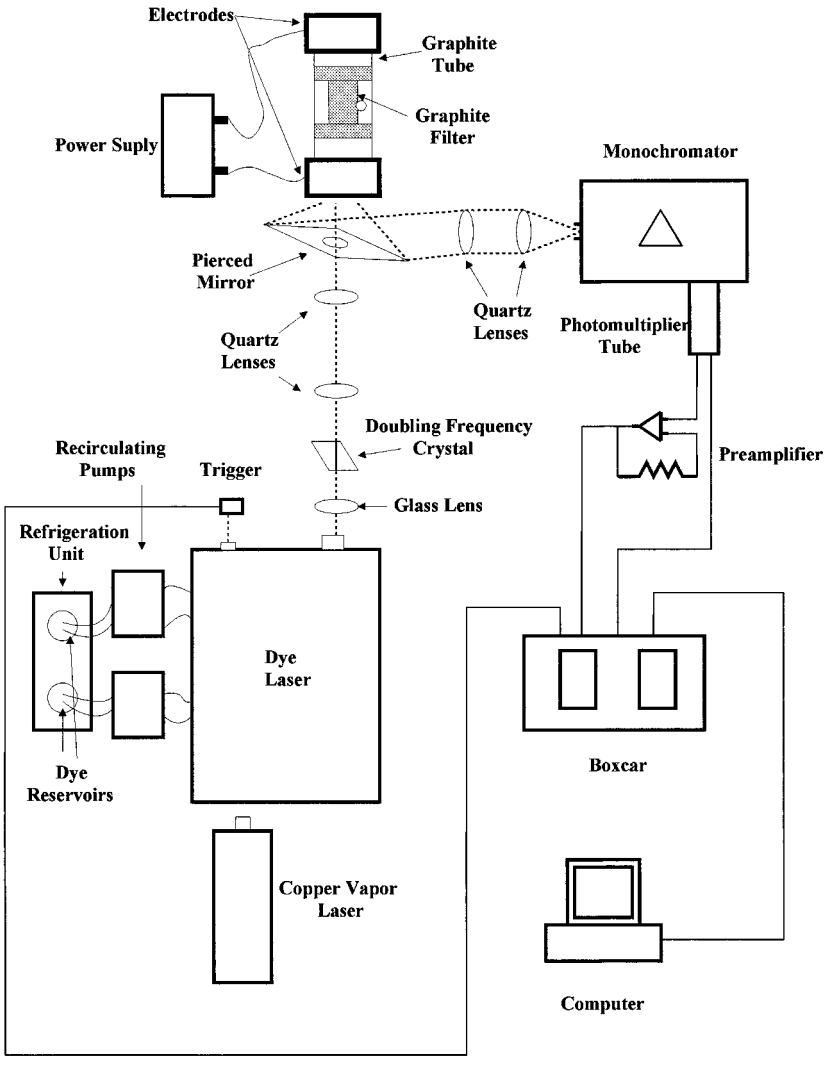

Fig. 1 Experimental setup.

to modify the flow of the argon gas during part of the heating cycle. The front-surface illumination scheme, comprising a pierced mirror, was used to collect the fluorescence radiation (405.7 nm). The detection system consisted of a monochromator (Model 340E, 340 mm focal length, F/n=5.9, 1200 grooves/mm holographic grating, Spex, Edison, NJ), a photomultiplier tube (PMT) (R928, Hamamatsu Corp. Bridgewater, NJ), a laboratory-made transimpedance amplifier and a boxcar integrator (SR245, Stanford Research, Sunnyvalley, CA), externally triggered with the signal from a fast photodiode fed with scatter radiation from the laser. The boxcar was adjusted to a 300 ns gatewidth and a 30 ns delay time. The signal was processed by a personal computer. Neutral density filters were used to avoid the saturation of the detector by the incoming radiation.

A laboratory-made spool shaped graphite filter[5] made of porous graphite (National Carbon Co., Cleveland, OH) was inserted into a pyrolytic graphite tube. The graphite filter was replaced eventually by a new one after the sensitivity decreased by more than 10% (after approximately 40 furnace firings). Pyrolytic graphite tubes with L'vov platforms were employed in part of the experiments.

An Eppendorf micropipett with a polypropylene tip was used to deliver blood and water samples into the furnace. A 25 □l microsyringe (Hamilton Co., Reno, NV) was used to measure lead solution volumes during the preparation of the laboratory prepared standards.

Reagents

All chemical reagents were of analytical grade and used without further purification. The 1000 ppm lead atomic absorption solution is from Aldrich Chemical Company Inc., Milwalkee, MA. Optima grade nitric acid used to clean the glass material is from Fisher Scientific, Fair Lawn, NJ. Deionized ultrapure filtered water (DIUF water) (Fisher Scientific, Fair Lawn, NJ) was used to prepare solutions as well as to clean glass material. NIST SRM 955a lead in blood was used as blood standards as well as blood samples to verify the validity of the method.

Procedures

Lead aqueous standard solutions were prepared by a sequential of dilutions of the 1000 ppm atomic absorption solution in a 0.01 M nitric acid solution prepared with deionized ultrapure water and Optima grade nitric acid. The standards were stored in PTFE bottles. Blood samples were 1/20 w/w diluted with a 0.01 M nitric acid solution. Aqueous and blood standards as well as the blank solutions (0.01 M nitric acid solution) were directly pipetted into the furnace.

Table 1 Furnace programs employed for the determination of blood by filter furnace (FF) and stabilized temperature platform furnace (STPF)

Step[a]	Temperature (˚C)/ramp time (s)/hold time (s) STPF	FF
Dry	80/20/30	80/20/30
Dry	180/20/20	180/20/20
Ash	(300 or 900)/10/35	300/10/40
Cool down[b]	—	20/10/30
Atomization[c]	1900/0/14	1900/0/14
Clean	2500/1/10	2500/1/10
Cool down	20/10/10	20/10/10

a. Argon flow except during the atomization step (stop flow).
b. Modified internal argon flow.
c. Signal record.

Results and Discussion

Laser-excited atomic fluorescence has been proved to be one of most promising analytical tools for ultratrace detection. High repetition rate lasers, like the copper vapor laser, have already been demonstrated to be an efficient excitation source to probe transient atomic populations generated in the graphite tube atomizer.[7,8] Unlike absorption based methods, the selective nature of the fluorescence minimizes interferences due other atomic species and most potential molecular interferents are greatly reduced. At the same time, the graphite tube is considered to be the best atomizer because of the increased concentration of the analyte vapor in the probed zone; however, there is also an increase in interferences due to the concentrated gas components in the small volume when complex matrices are measured. The potential of the filter furnace (FF) technique to minimize this type of matrix interference was demonstrated by Katskov[6] when absorption signals of lead and cadmium, in diluted whole blood, obtained with FF technique were similar to those obtained with SPTF and Zeeman background correction.

In order to illustrate the usefulness of the method, the

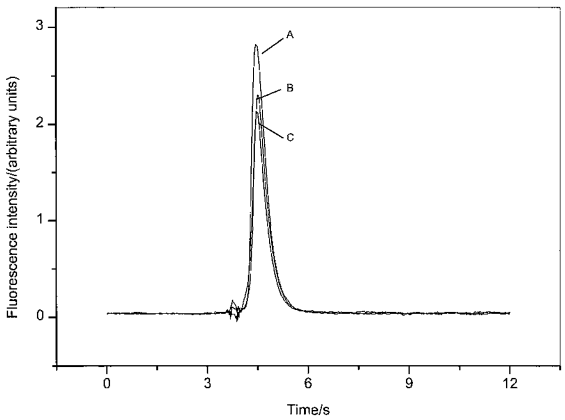

Fig. 2 Fluorescence temporal profiles of lead using stabilized platform temperature furnace (SPTF) technique. (A) 6.8 ng g^{-1} lead aqueous standard; (B) 6.8 ng g^{-1} lead blood standard using 900˚C temperature ashing step; (C) 6.8 ng g^{-1} lead blood standard using 300˚C temperature ashing step.

Table 2 Effect of the volume of blood samples deposited in the filter furnace on the recovery and reproducibility of lead fluorescence signal

	Volume of sample[a]		
	1 l	2 l	5 l
Recovery (%)[b]	98.3	94.1	88.5
Reproducibility (%)[b]	6.8	11.3	13.8

a. Lead in blood (NIST 955a-1) prepared using a 1/20 w/w sample/0.01 M nitric acid solution.
b. Each result is based on 10 replicates.

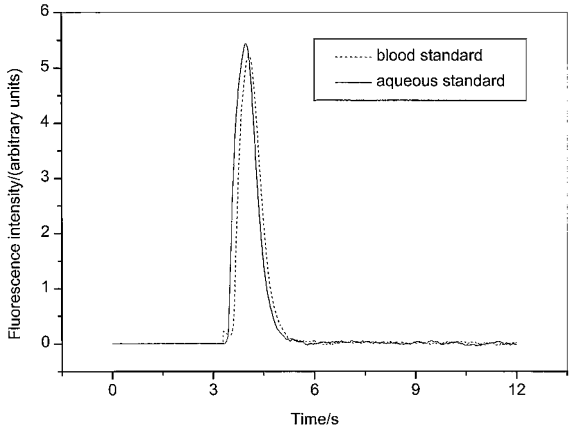

Fig. 3 Fluorescence temporal profiles of lead using filter furnace (FF) technique using 15.3 ng g^{-1} lead aqueous standard (—) and 15.3 ng g^{-1} lead blood standard (---).

filter furnace technique was compared with the stabilized temperature platform furnace (STPF) technique for the determination of lead in whole blood by laser atomic fluorescence. Atomization parameters for STPF and FF are shown in Table 1. A slow two part drying step was employed to avoid splattering of the sample in both cases. For SPTF, two different ashing steps were evaluated. First, a 900°C temperature was used to completely ash the blood matrix prior to atomization. In this case, the Pb signal of a 6.8 ng g^{-1} blood standard was approximately 20% smaller than the Pb signal of 6.8 ng g^{-1} water standard (Fig. 2). This difference can be accounted by losses of lead by vaporization and/or by the formation of lead organic compounds at relatively moderate temperatures.[3] To overcome those problems, a lower ashing temperature (300°C) was used. However, in this case the lead signals for 6.8 ng g^{-1} Pb aqueous standards were 27% higher than for 6.8 ng g^{-1} Pb blood standard (Fig. 2). The difference in intensities in this case can be attributed mainly to the attenuation of the excitation laser radiation as well as the emitted fluorescence by the smoke released and to fluorescence quenching of excited species by the high concentration of molecular species in the analytical zone during atomization.[4]

With the filter furnace technique, several heating programs were evaluated. The best program (Table 1)

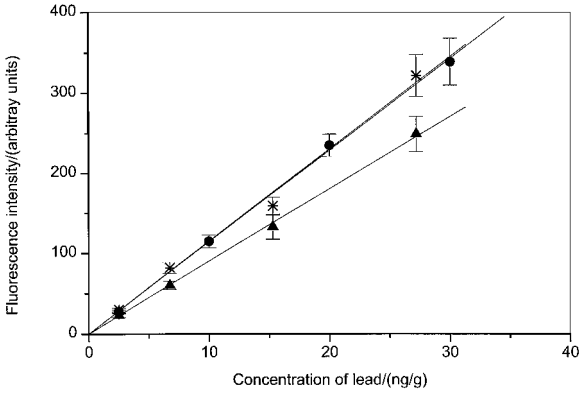

Fig. 4 Calibration curves of lead. (●) Aqueous standards using filter furnace technique; (✻) blood standards using filter furnace technique; (▲) blood standards using stabilized temperature platform furnace technique. Blood calibration curves were made using NIST samples diluted 1/20 w/w with water.

included a pre-atomization cycle with two drying steps and a low temperature ashing step (300°C), to avoid the problems related to the use of the high temperature pre-atomization discussed above. A cool down/cleaning step, immediately before atomization of the sample, was used in order to allow dissipation of smoke from the analytical zone resulting from imperfections of the fitting of the filter in the graphite tube. In order to increase the rate of dissipation of smoke, a modification of the internal argon flow of the HGA 400 furnace during the cool down was made. This modification consisted of an electrical bypass, inserted in the tubing on one side of the internal argon flow. The bypass was manually turned on at the beginning of the cool down step in order to stop one side of the internal argon flow, and turned off to restore the normal flow right before the atomization of the sample. This modification of the internal flow improved the reproducibility of the results.

Laser-excited atomic fluorescence using a copper vapor laser has proven to be a very sensitive method for the determination of lead where a limit of detection of 0.1 fg has been reported in a previous study made in our laboratory[4], in contrast to 5 pg that can be achieved by graphite furnace atomic absorption spectrometry.[2] Taking advantage of this high sensitivity, the amount of blood deposited in the filter furnace was reduced to 1 □l to minimize the amount of organic matter and the amount of fumes released. The reduction of the volume of blood sample improved the recovery of lead (in percent) when compared to higher volumes of sample (Table 2). Volumes above 10 □l were not evaluated because in this case, part of the sample was expelled from the filter cavity during the heating program. The reproducibility of the results was also improved with the reduced sample volume as can be seen in Table 2. Reproducibility around 6.0% were found for 1, 2 and 5 □l when experiments with 6 ng g^{-1} lead aqueous standards were performed. In addition, an increase in the lifetime of the graphite filter was also observed when

Table 3 Determination of lead in reference materials[a] by FF-LEAFS

NIST sample	Nominal concentration of lead/ng g^{-1}	Experimental result by calibration curve method[b] (recovery %)
955a-1	50.1 □ 0.9	54.3 □ 4.5 (108.38)
955a-2	135.3 □ 1.3	129.8 □ 8.7 (95.93)
955a-3	306.3 □ 3.2	296.2 □ 19.3 (96.7)
955a-4	544.3 □ 3.8	537.5 □ 39.5 (98.75)

a. 1 l of sample introduced in the furnace.
b. Each experimental result is based on 10 repetitions.

working with the minimized amount of sample.

Fluorescence–time profiles of lead in both aqueous and the blood standard of the same concentration can be seen in Fig. 3. The figure shows that signals from both standards are similar, indicating that interferences of the blood matrix in the analyte signal were successfully remedied. Calibration curves (Fig. 4) constructed using blood standards and water standards are basically identical indicating excellent correlation between them. This correlation means that our method enables a precise determination of lead in blood samples using a calibration curve made with aqueous standards. A calibration curve made using SPTF technique is also shown in Fig. 4 for comparison.

The validity of the method was evaluated by analyzing blood samples with a certified concentration of lead (SRM 955a). Results displayed in Table 3 show that very good recoveries of lead were obtained by using the proposed method.

In conclusion, the possibility of the use of calibration curves made with aqueous standards make the sensitive filter furnace laser atomic fluorescence spectrometry a method that has the potential to simplify the determination of lead present in complex matrices.

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References

1. D. Priestner, L. A Sternson and A. J. Repta, *Anal. Lett.*, **14(B15)**, 1255 (1981).
2. W. Slavin (ed.), "*Graphite Furnace AAS: A Source Book*", p. 15, Perkin-Elmer, Ridgefield, 1984.
3. D. L. Tsalev, V. I. Slaveikova and P. B. Mandjukov, *Spectrochim. Acta Rev.*, **13**(3), 225 (1990).
4. E. P. Wagner, B. W. Smith and J. D. Winefordner, *Anal. Chem.*, **68**, 3199 (1996).
5. D. A. Katskov, R. I. McCrindle, R. Schwarzer, P. J. J. G. Marais, *Spectrochim. Acta*, **50B**, 1543 (1995).
6. D. A. Katskov, P. J. J. G. Marais and P. Tittarelli, *Spectrochim. Acta*, **51B**, 1169 (1996).
7. R. Q. Aucélio, B. W. Smith and J. D. Winefordner, *Appl. Spectrosc.*, **52**, 1457 (1998).
8. R. Q. Aucélio, V. N. Rubin, B. W. Smith and J. D. Winefordner, *J. Anal. At. Spectrom.*, **13**, 49 (1998).

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