Scientia Iranica, Vol. 12, No. 3, pp 324 32

SCIENTIA IRANICA

Research Note

In-Situ Digestion of Serum Samples in Graphite Furnace Prior to Determination by ETAAS

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An accurate in-situ digestion method was developed for this approach to separate the traces of Pb, Mn and Zn from their concomitant in serum samples, prior to determination by ETAAS. Pd was used as a modifier to stabilize the analyte from decomposition at 900-1000 (°C). Stabilization was much more effective if the mixture modifier and digested sample were first pre-pyrolyzed on the furnace prior to the atomization. Determination of Pb, Mn and Zn in serum at a concentration of 0.1 ppb-1.0 ppm was performed by this method. Percent relative standard deviations for seven replicates in measurements were found to be 3.4, 3.1 and 4.0, respectively. The accuracy of technique was established by analyzing standard reference materials. The results for determination of the tested elements were within the range of certified values.

INTRODUCTION

Determination of trace elements in blood and body fluids are, increasingly, being considered as useful and important tests in medicine, because the levels of some elements can be related to various pathological conditions in man. Therefore, the accurate and reproducible determinations of trace elements in biological samples are of vital importance [1-8].

However, analysts encountered many problems in the determination of trace elements in real samples, when solving them were necessary for obtaining reliable results. The main problems are the difficulties in achieving adequate S/N, gaining the required selectivity for trace components at much higher concentrations of concomitant, obtaining sufficiently pure chemicals and avoiding contamination of samples during chemical pretreatment processes. Among various analytical techniques with sufficient attributes for reducing these difficulties and solving the above problems, Electro Thermal Atomic Absorption Spectrometry (ETAAS) has been proved to be the most suitable for this purpose. Moreover, the sample volume requirement is in the submicroliter range and pretreatment would be easier in ETAAS. However, the direct determination of trace elements in biological samples is still difficult, even using ETAAS, because not only are their concentrations near or below the detection limit of this technique, but also, because the matrix of these samples may cause serious interference. Preconcentration and separation of analyte from interfering concomitant by digestion and chemical pretreatment may solve these problems and enable a more sensitive and accurate determination [4,6,9].

However, the ex-situ wet digestion, which achieves complete charring of organic material, may introduce some contamination and dry ash may lead to loss of any volatile species [2,4-7,10]. Therefore, it is necessary to develop a suitable digestion method, with minimal sample pre-treatment, to separate the traces of analyte from concomitant in serum samples prior to determination by ETAAS. The use of a modifier is also necessary to permit a high enough pyrolysis temperature in order to remove the bulk of concomitants during thermal pretreatment of the samples without losing any analyte prior to the atomization step [11].

Moreover, there are a number of requirements necessary to make the application of modifiers generally acceptable in ETAAS:

i) The analyte element should be stabilized to as high a pyrolysis temperature as possible, to allow volatilization of the bulk of concomitant;

ii) The modifier should be applicable to as many elements as possible;

iii) The modifier should be available at a high level of purity and not contain analyte elements in any measurable concentration;

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iv) The modifier should not produce excessive background attenuation around the wavelength of the analyte element;

v) The modifier should not markedly reduce the lifetime of the graphite tubes [12].

Among all of the proposed modifiers, Pd fulfils all the requirements [13]. Stabilization to high enough pyrolysis temperatures can be obtained. Pd is certainly not a frequently determined element and it can be obtained at high levels of purity. It does not reduce the furnace lifetime and does not produce any excessive background attenuation.

In this work, an accurate in-situ digestion method is developed, with minimal sample pre-treatment to separate the traces of Pb, Mn and Zn from concomitant in microliters of injected serum samples directly inside the graphite furnace prior to determination by ETAAS. Palladium is used as the analyte and matrix modifier for removing the bulk of concomitants during thermal pre-treatment and preventing the escape of volatile analyte elements prior to the atomization step.

EXPERIMENTAL

Instrumentation

A Perkin-Elmer 503 Atomic Absorption Spectrometer with a deuterium arc background correction, equipped with a HGA-2100 furnace controller, has been used in this research.

Collection of Blood Samples

After clotting, the serum was separated by centrifuge and was transferred into a clean tube. Then, the sample was kept frozen until the time of analysis. In this procedure, serum is directly injected into the graphite furnace before digestion.

Reagents

Ultrapure water was obtained by passing distilled water through a milli-Q ion exchange and membrane filtration system. All acid used, namely, HNO_3, HCl and H_2SO_4, were of Merck ultrapure quality and all were diluted by milli-Q water. Sodium chloride solutions were prepared from a high purity grade or analytical reagent. A stock Pd solution (3000 ppm) was made by dissolving high purity grade $PdCl_2$ in 1% of HNO_3 and was diluted to a 100 ppm working solution with milli-Q water. All analyte standard solutions were prepared in 1% HNO_3 or 1% HCl by diluting stock solutions of 1000 ppm (BDH) standard solution for AAS. All solutions were stored in high density polyethylene containers previously soaked in 1% HNO_3 for a long time.

RESULTS AND DISCUSSION

Thermal Stabilization by Using Pd

In general, it is desirable to use a pyrolysis temperature as high as possible in order to remove the matrix interference. Pyrolysis curves for Pb are shown in Figure 1 using different modifiers proposed in literature. The results showed that 100 ppm Pd was the best for stabilizing the traces of Pb from decomposition at 900°C.

In the absence of a modifier, volatilization loss occurs if the sample is pyrolyzed above 400°C. The addition of Pd leads to the stabilization of Pb to a much higher temperature (900°C). The Pd modifier increases the maximum pyrolysis temperature by about 500°C [14], as shown in Figure 2. Stabilization occurs through adsorption of analyte by the pre-pyrolyzed palladium. The analyte remains adsorbed on the reduced palladium until the halide matrix has volatilized during the pyrolysis stage. It is claimed [15] that on further heating, the analyte is embedded in molten palladium and diffuses out during the atomization stage.

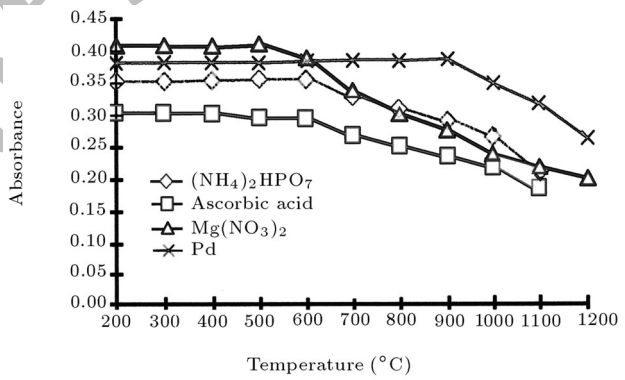

Figure 1. The stabilizing effect of different modifiers on pyrolysis curves of 40 ppb Pb in a solution containing 0.15 M NaCl and 3% HNO_3.

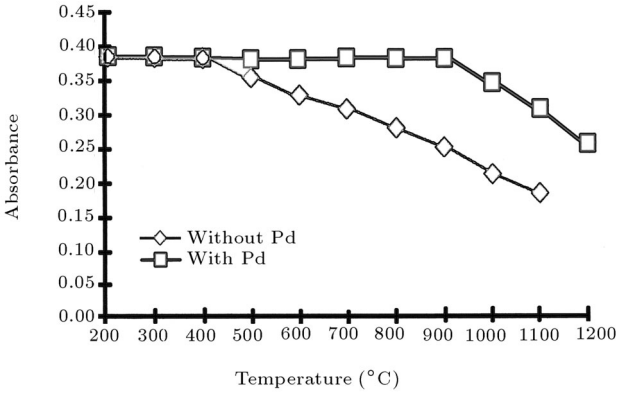

Figure 2. Pyrolysis curves for 40 ppb Pb, with 100 ppm Pd and without Pd in a solution containing 0.15 M NaCl and 3% HNO_3.

Table 1. Thermal programs for determination of traces of Pb, Zn and Mn in serum sample by in-situ digestion.

		Pb		Zn		Mn	
	Step	**Temp. (°C)**	**Time (sec)**	**Temp. (°C)**	**Time (sec)**	**Temp. (°C)**	**Time (sec)**
Cycle (1)	Drying	50 → 500	80	50 → 500	80	50 → 500	80
	Ashing	900	80	900	80	1200	80
Cycle (2)	Drying	500	70	500	70	500	70
	Ashing	900	70	900	70	1200	70
	Atomization	2300	5	2300	5	2600	5
	Cleaning	2600	5	2600	5	2600	5

The pyrolysis curves for Mn and Zn traces in these solutions were also obtained, which are shown in Figures 3 and 4, respectively.

In-Situ Digestion Procedure and Thermal Program Optimization

15 μl serum samples, which were diluted with an equal volume of 1% of Triton X-100 solution, 15 μl of 3% HNO_3 and 15 μl of Pd (100 ppm), were introduced to the furnace via a GILSON micropipette. When the spiked samples, for standard addition experiments, were carried out, no more acids were added, as the standards were already prepared in 3% HNO_3 to avoid splashing of the high injected sample volume during the drying process.

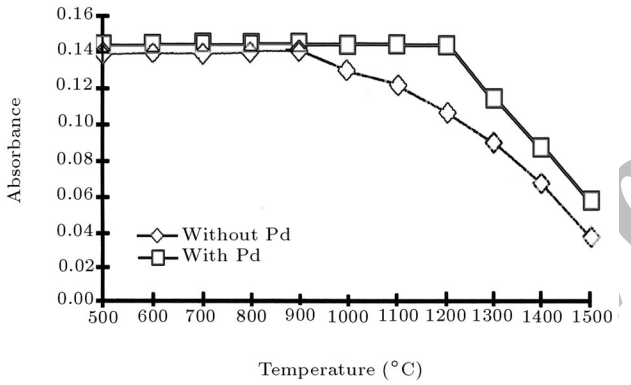

Figure 3. Pyrolysis curves for 10 ppb Mn, with 100 ppm Pd and without Pd in a solution containing 0.15 M NaCl and 3% HNO_3.

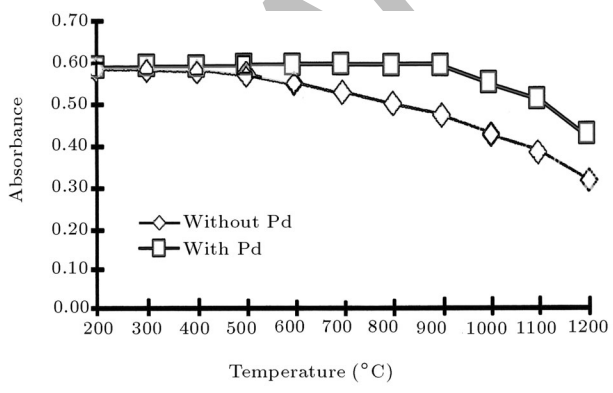

Figure 4. Pyrolysis curves for 40 ppb Zn, with 100 ppm Pd and without Pd in a solution containing 0.15 M NaCl and 3% HNO_3.

The standard solutions of 0, 10, 20, 30 and 40 ppm Zn, Pb and Mn in 3% HNO_3 were measured by using the optimal instrumental conditions. The furnace thermal programs for in-situ digestion (cycle 1) and conventional atomization (cycle 2) are shown in Table 1.

The furnace thermal program included two main steps, the first one was the in-situ digestion and pyrolysis step (cycle 1 in Table 1), followed by a conventional thermal step for measurement of elements (cycle 2) [15].

The experimental results have shown that stabilization and digestion are much more effective if, prior to the atomization stage, Pd and the sample are first pre-pyrolized on the furnace platform at 900°C. These findings are in agreement with those of Qiao et al., which are reported in the literature [14,15]. Therefore, the samples were in-situ digested and the analyte was dissolved in the droplets of molten Pd (or possibly its oxide), accompanied by compound formation, as discussed by these researchers [14,15].

The results for measurement of Pb, Zn and Mn in serum samples by the in-situ digestion technique, followed by ETAAS, are shown in Table 2.

The figures of the merits of the technique are shown in Table 3.

Table 2. Average Pb, Zn and Mn concentrations in RSM serum by in-situ digestion.

Element	**Concentration (RSM Serum)**
Pb	4.051 ± 0.139 ppb
Zn	0.832 ± 0.034 ppm
Mn	1.0546 ± 0.048 ppb

Table 3. Figures of the merits of the technique.

Element	m_o(pg)[1] Blank	m_o(pg)[1] Serum	D.L[2]	%RSD $n = 7$
Pb	5.892	5.454	0.339 ppb	3.431
Zn	1.274	1.571	0.912 ppm	4.086
Mn	4.714	5.156	0.168 ppb	3.104

1 m_o, characteristic mass $\frac{(\text{volume of sample, } \mu\text{l})(\text{conc. of sample, pg}/\mu\text{l})(0.0044)}{\text{Absorbance}}$.

2 D.L. Detection Limit $3s_b/m$, s_b standard deviation of blank, m slope of calibration curve.

The consistency, in slope, of the standard calibration curve and that of the serum spike curve indicated that in-situ digestion could eliminate a high amount of matrix during the ashing stage. The results of these experiments are shown for Zn in Figure 5. The results for recovery are also good proof for this verification, as shown in Table 4.

The same results were obtained for Pb and Mn as well. The slopes of calibration curves and corresponding correlation coefficients are shown in Table 5.

EX-SITU DIGESTION PROCEDURE

50 μl of 50% nitric acid was added to a 250 μl of plasma and was diluted to 500 μl with high purity water. The tube was stoppered with a polyethylene cup and the sample was vigorously agitated for 30 sec and centrifuged at 3000 rpm [16].

The protein-free samples, with an equal volume of 1% solution of Triton X-100 + 15 μl of 1% nitric acid, were introduced to the furnace and the temperature program was performed as shown in Table 6.

Table 4. Results for measurement of Zn in spiked serum and blank samples after in-situ digestion.

	Serum	Blank	
Zn con./ppb	Abs.	Abs.	% Recovery
0	0.280	0.00	
10	0.420	0.141	99.29
20	0.556	0.278	99.28
30	0.689	0.412	99.27
40	0.824	0.549	99.08
Slope & correlation coefficient	1.357×10^{-2} $r = 0.999$	1.369×10^{-2} $r = 0.999$	

The results for measurement of Pb, Zn and Mn in an SRM serum sample by an ex-situ digestion technique are shown in Table 7.

CONCLUSION

The comparison of in-situ and ex-situ digestions on an SRM serum sample (from the International Atomic Energy Agency) shows that the deproteinization in the ex-situ digestion procedure by precipitation of proteins causes a loss of trace elements that may be linked to the protein. The results showed much suppression in the measured value as compared to the results for the same samples obtained by the in-situ digestion technique. This finding has been proved by using FAAS for Zn measurement in the serum compared with both in-situ and ex-situ techniques.

The result showed the range of concentration

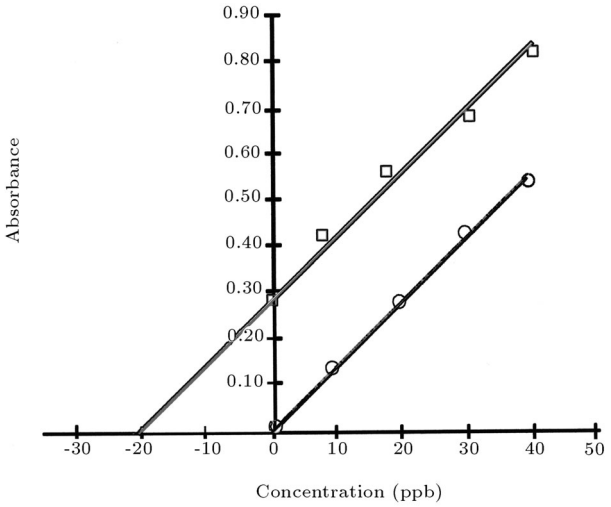

Figure 5. The calibration curves for Zn content, spiked to the serum samples (□), the same spiked to the blank (○), measured by proposed in-situ digestion technique.

Table 5. Results for measurement of spiked serum and blank by Pb and Mn standard solutions after in-situ digestion.

Element	Slope and r (Spiked to Serum)	Slope and r (Spiked to Blank)	% Recovery
Pb	$9.48 \times 10^{-3} r = 0.999$	$9.70 \times 10^{-3} r = 0.999$	98.69
Mn	$1.11 \times 10^{-2} r = 0.999$	$1.13 \times 10^{-2} r = 0.999$	98.20

Table 6. Thermal program for determination of Pb, Zn and Mn in the serum sample by ex-situ digestion.

	Pb		Zn		Mn	
Step	**Temp. (°C)**	**Time (sec)**	**Temp. (°C)**	**Time (sec)**	**Temp. (°C)**	**Time (sec)**
Drying	50 → 200	80	50 → 200	80	50 → 500	80
Ashing	400	80	400	80	900	80
Atomization	2100	4	2100	4	2500	4
Cleaning	2600	4	2600	4	2600	4

Table 7. Average SRM serum Pb, Zn and Mn concentrations after ex-situ digestion.

Element	Concentration (SRM serum)	%RSD	%Recovery
Pb	2.006 ± 0.084 ppb	4.000	95.69
Zn	0.742 ± 0.037 ppm	4.986	98.92
Mn	0.912 ± 0.036 ppb	3.947	98.24

to be 0.1ppb-1ppm in the serum sample for these elements. Pd shows a high capability as modifier to stabilize the analyte from decomposition at 900-1000°C. The accuracy of the method was verified using International Atomic Energy Reference Standard Material (SRM). The results for the tested elements were consistent with the certified values in the range of accepted RSD.

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