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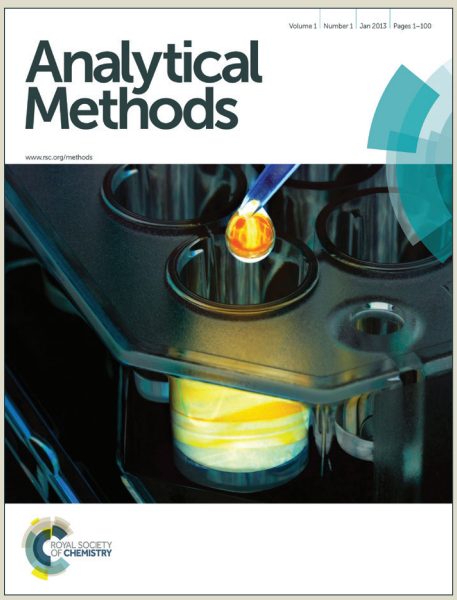

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Determination of aluminum and silicon in bovine liver by graphite furnace atomic absorption spectrometry after dissolution with tetramethylammonium hydroxide

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Abstract

Studies suggest that silicic acid or silica can reduce the oral absorption and increase the excretion of aluminum thus protecting the organism against the adverse effects induced by this metal. Therefore, the simultaneous or concomitant determination of Al and Si in biological samples is of significance. In this study a method for sample treatment and the determination of both Al and Si by graphite furnace atomic absorption spectrometry (GFAAS) in animal tissue was developed. Sample dissolution with tetramethylammonium hydroxide (TMAH) proved to be suitable for the determination of both elements. Because Si enhances the Al signal during atomization, this element acted as a chemical modifier for the determination of Al. For the determination of Si, palladium nitrate was the most suitable modifier. The use of Zr as a permanent modifier minimized the tube degradation caused by TMAH. The limits of detection were 5.8 μg L^{-1} for Al and 29.0 μg L^{-1} for Si, and the recoveries in spiked samples were between 97-112%. The method was validated against bovine liver standard reference materials (SRM 1577b and 1577c), and the obtained concentrations agreed with the certified values.

1. Introdution

Aluminum and silicon are two of the three elements most abundant in the lithosphere. In spite of this, during human evolution these elements seemed to be excluded from biochemical processes. Exposure to Al and Si has been increasing due to modern technologies based on both elements [1].

Aluminum is a nonessential element to which humans are often exposed. A particular form of exposure is associated with infusion therapy to replace the hydrolyte balance, or parenteral nutrition, where elevated volumes of solutions are administered directly into the bloodstream of the patient [2]. Although Al may be considered harmless to a healthy organism when orally ingested, in cases where the administration is parenteral and the organism is debilitated or its excretory functions are disabled, this metal is potentially toxic [3]. Under these circumstances, Al toxicity is related to bone diseases [2], Alzheimer's disease [4-6], Amyotrophic Lateral Sclerosis [7] and alterations in the hematopoietic system [8].

The biological role of Si is even more unknown than that of Al; nevertheless, it is generally considered an element of low toxicity [1]. Considerable amounts of this element are found in

various food products and in all natural waters [9]. The nutritional interest in Si has been focused on its beneficial effects on the formation of collagen and glycosaminoglycans or functions that could influence bone formation, cardiovascular health and wound healing. Despite the numerous signs of Si deficiency that have been described, it is not generally accepted as an essential nutrient for higher animals and humans [10].

With the growing interest in the biological effects of aluminum and silicon, the development of precise and accurate analytical methods for measuring these elements in biological materials is important. Analytical techniques commonly employed for the measurement of silicon and aluminum in biological samples are inductively plasma atomic emission (ICP-OES), inductively plasma atomic mass spectrometry (ICP-MS) [11-13] and graphite furnace atomic absorption spectrometry (GFAAS) [14, 15]. Among these, GFAAS provides the highest sensitivity and reliable results for the measurement of silicon and aluminum in biological samples [16] by the existence of a pyrolysis step that destroys matrix constituents before the measurement step.

Some studies suggest that silicic acid or silica can reduce the oral absorption and/or increase the excretion of aluminum and therefore protect the organism against the adverse effects induced by this metal [13, 17, 18]. This protective effect would be credited to the formation of hydroxyaluminosilicates.

Biological samples often require extensive pretreatment prior to analysis, and acid digestion at elevated temperatures is the method of choice for the determination of most elements. An alternative to this is the solubilization of the sample with tetramethylammonium hydroxide (TMAH), a strong and hydrosoluble base [19]. The sample is mixed with a small volume of a TMAH solution in an open vial and usually requires no heating. Samples treated with TMAH are suitable for analysis by GFAAS because this technique does not require dissolution or total digestion of the sample [20].These advantages has been firstly demonstrated by Silva et al [21]. GFAAS has since then been employed in the determination of volatile elements in biological tissues after treatment with TMAH [19-22].

This work aimed to establish a method for the sample preparation of biological tissues that allows the determination of Al and Si by GF-AAS in the same prepared sample. Bovine liver was used as a model tissue due to its high fat content. Acid and alkaline dissolution with TMAH were tested and compared for the determination of these elements.

2. Materials and methods

2.1. Instrumentation/Procedure

All of the measurements were carried out using an ANALYTIK Jena AG (Jena, Germany) model ZEEnit 600 atomic absorption spectrometer equipped with a transversely heated graphite atomizer, a MPE 60z auto-sampler and a transversal Zeeman-effect background correction system. The integrated absorbance (peak area) values were used for evaluating the signal. Al and Si hollow cathode lamps from Analytik Jena were employed as the radiation sources. Argon 99.996% (White Martins, São Paulo, Brazil) was used as the purge gas. Atomization was made on pyrolytic coated graphite tubes (without integrated platforms) from Analytik Jena. The instrumental parameters and operational conditions were those recommended by the manufacturer. The furnace Al and Si temperature programs as well as the conditions for their measurements are shown in Table 1.

For the Zr tube covering, the tube was treated with $ZrCl_4$ by applying 40 µL of a 500 mg L^{-1} Zr solution onto the furnace and submitting the tube to a specific temperature program, which is also shown in Table 1. This procedure was repeated 25 times to obtain a deposit of 500 µg of Zr as a permanent modifier.

Table 1: Instrumental parameters for the determination of Al and Si in bovine liver by GFAAS and furnace treatment with Zr.

Parameter	Al	Si	Zr treatment
Wavelength (nm)	309.3	251.6	_
Sample volume (µL)	20	20	
Modifier (µL)[1]	Si (5)	Pd $(NO_3)_2$ (5)	Zr (40)
Heating temperature program (°C) (ramp time (s), hold time (s))			
Drying 1	90 (5, 20)	90 (5, 20)	90 (7, 15)
Drying 2	105 (3, 20)	105 (3, 20)	140 (10, 15)
Drying 3	110 (2, 10)	110 (2, 10)	_
Pyrolysis	1500 (250, 10)	1200 (250, 10)	1000 (100, 10)
Atomize	2600 (FP, 4)	2600 (FP, 3)	2000 (FP, 5)
Cleanout	2650 (500, 4)	2650 (500, 4)	_

[1] Si: 15 mg L^{-1}; Pd $(NO_3)_2$ 2 g L^{-1}; $ZrCl_2$: 500 mg L^{-1}

2.2. Reagents

All of the reagents were of analytical grade, and all of the solutions were prepared with distilled and deionized water that was further purified by a Milli-Q high purity water device (electrical resistivity of 18.0 MΩ cm) (Millipore, Bedford, USA). To avoid Si and Al contamination from glass, only plastic materials were used. All of the laboratory equipment (polypropylene pipette tips, polyethylene volumetric flasks, etc.) was immersed for at least 48 h in a 10% (v/v) HNO_3/ethanol solution and washed with Milli-Q purified water shortly before use. To reduce contamination from the air, all of the steps in the preparation of the samples and reagents were conducted on a Class 100 clean bench. The TMAH (solution containing 250 g L^{-1}) and concentrated nitric acid used in this study were supplied by Sigma-Aldrich (St Louis, USA) and Merck (Darmstadt, Germany), respectively. Nitric acid was further sub-boiling distilled in a Berghof Teflon apparatus (Eningen, Germany). Calibration solutions containing 50 µg L^{-1} of Al and Si were prepared by adequate dilutions of a 1000 mg L^{-1} stock solution (NIST). The chemical modifiers used in the measurement of the elements by GFAAS were palladium nitrate (2 g L^{-1}) (Fluka, Switzerland), magnesium nitrate (1 g L^{-1}) (Merck), calcium chloride (100 mg L^{-1}) (Merck), and zirconium chloride (500 mg L^{-1}) (Merck).

2.3. Calibration

Calibration curves were established, and the integrated absorbance values were obtained by injecting 20 µL of standard solutions containing 0, 10.0, 20.0, 30.0, 40.0 and 50.0 µg L^{-1} of Al or 20 µL of standard solutions containing 0, 100.0, 200.0, 300.0, 400.0 and 500.0 µg L^{-1} of Si into the furnace. For Si, an addition

calibration method was utilized. The method was validated by the determination of the following operational characteristics: linearity, detection limit, precision and accuracy. The method linearity was evaluated by six-point calibration curves performed on three different days for each analyte. The limits of detection (LOD, μg L^{-1}) were calculated from the equation LOD = 3.3 × S_a/b, where S_a was the intercept standard deviation and b was the slope [23]. Three replicates were measured unless otherwise stated, and all of the measurements were based on the integrated absorbance values. The precision was expressed by the variation coefficients (expressed as RSD) of the results obtained in triplicate for three different concentrations of each analyte (n=9). Accuracy was evaluated as a percentage of recovery obtained from analyzing ~~of~~ Standard Reference Materials. The Bovine Liver SRM 1577b and 1577c from the National Institute of Standards and Technology (NIST, USA) were analyzed for Al and Si, respectively.

2.4. Sample treatment

Bovine liver was used for testing different sample treatments prior to the determination of the elements by GF-AAS. Samples were weighed in amounts ranging from 0.2 to 0.3 g and placed in plastic flasks previously decontaminated. In each flask, different amounts of concentrated nitric acid (1, 2, 3 and 5 mL) or 250 g L^{-1} HTMA (0.5, 1 and 2 mL) were added. The samples were made in duplicate, and in one of them Al and Si were added to obtain final concentrations of 100 and 300 μg L^{-1}, respectively. The samples were kept in water bath at 100 °C for one hour and then held for 24 h at room temperature (25 °C). A blank test was also carried out for each reagent and volume. After this procedure, the volume was completed to 10 mL with ultrapure water, and the Si and Al contents were determined by GFAAS determine the percent recovery for each procedure as well as the optimum amount of reagent.

2.5. Analysis of the standard reference materials

The standard reference materials Bovine Liver SRM 1577b and 1577c from the National Institute of Standards and Technology (NIST, USA) were analyzed for their Al and Si contents, respectively. For this, the selected sample treatment was employed, i.e., dissolution with TMAH. In this sample treatment, 0.05 g (dry mass corresponding to 0.25 g wet weight) were weighed and dissolved with 0.5 mL of TMAH according to the procedure previously described.

3. Results and discussion

3.1. Chemical modifiers and temperature programs

Si increases the graphite furnace Al signal. According to the literature records, in samples containing these two elements the concentration of Al can be overestimated by up to 50% [24]. However, due to this effect, Si can be used as a chemical modifier for the determination of Al by GFAAS. Schneider and C. Exley [24] have hypothesized that the presence of Si reduces Al carbide formation during the atomization step. It was shown that this influence is saturated at Si concentrations above 14 mg L^{-1}; therefore, a Si concentration of 15 mg L^{-1} was chosen to equally match the effect in all of the samples and standards measured. Figure 1 shows the pyrolysis and atomization curves for Al (50 g L^{-1}) with the use of a 15 mg L^{-1} Si solution as a modifier, and a significant increase in the absorbance can be observed with this approach. The highest absorbance occurred at a pyrolysis temperature of 1500 °C and atomization at 2600 °C; thus, these temperatures were selected for all of the Al measurements. This figure also shows that the TMAH did not interfere with the Al absorbance because the pyrolysis and atomization curves were not significantly different with and without its addition.

The behavior of Si in the graphite furnace is complex and not fully understood [16, 25, 26]. Due to its ability to produce refractory carbides and volatile oxides during the atomization cycle in the graphite furnace, the determination of Si by GFAAS can undergo a memory effect, matrix interference and have a relatively low sensitivity [27]. Attempts to overcome these problems usually involve the use of graphite tubes coated with carbides of metals and the use of chemical modifiers [16] such as palladium that reduces losses due to volatilization during pyrolysis [28].

Figure 2 shows the signals generated by Si measured by GFAAS with the use of some chemical modifiers. Without the use of any modifier, the integrated absorbance was found to be very small and wide (Figure 2a). $CaCl_2$ improved the signal definition, but the background signal was high (Figure 2b). The use of combined modifiers such as $Pd(NO_3)_2/CaCl_2$ (Pd+Ca) (Figure 3c) and $Pd(NO_3)_2/Mg(NO_3)_2$ (Pd+Mg) (Figure 2d) neither led to an increase of the signal nor a reduction in the background. Although these chemical modifiers have been tested and satisfactorily used by some authors [16, 28, 29], in our case Pd rendered the best defined absorption peak of Si. The most significant signal improvement occurred with the use of $Pd(NO_3)_2$ alone (Figure 2e), where the signals were high and narrow.

Figure 3 shows the pyrolysis and atomization curves for Si, and a significant increase in the absorbances caused by the use of a Pd modifier can be observed. The highest absorbances were at temperatures of 1200 °C and 2600 °C for pyrolysis and atomization, respectively. The presence of TMAH interfered by decreasing the Si absorbance; however, this interference was minimized by using the standard addition method for making the calibration curves. Another important factor is that palladium nitrate precipitates when mixed with the alkaline solution of TMAH. To avoid this inconvenience, the modifier solution was pipetted separately from the sample with a capillary washing step between the injections.

The limit of detection (LOD) for Al was 5.8 μg L^{-1} when Si was used as a modifier. The LOD for Si with Pd as a modifier was 5.6 μg L^{-1} for the standard calibration curve method and 29.0 μg L^{-1} for the calibration curve made using a standard addition method. The characteristic mass (mŋ0) was 26 pg and 109 pg for Al and Si, respectively. The results obtained for linearity, LOD and precision are summarized in Table 2. The linearity data were validated using an analysis of variance (ANOVA), which demonstrated significant linear regression and no significant linearity deviation ($P < 0.05$). The low % RSD obtained for all samples indicated good precision and repeatability for the method.

Table 2: Figures of merit

Parameters	Al [a]	Si [a]
Analytic range (μL L^{-1})	0-50	0-500
Slope ± standard deviation (L μg^{-1})	0.0034 ± 1.9 x 10^{-4}	0.0008 ± 2.9 x 10^{-5}
Intercept ± standard deviation	0.0231 ± 6.0 x 10^{-3}	0.1255 ± 7.0 x 10^{-3}
Confidence limit of slope[b]	0.0028 to 0.0039	0.00073 to 0.00089
Confidence limit of intercept[b]	0.0066 to 0.0396	0.1059 to 0.1452
Correlation coefficient (r)	0.9934	0.9975
LOD (μg L^{-1})	5.8	29.0
mŋ0 (pg)	26	109
Repeatability RSD (n=9)	4.5	4.2

[a] Data obtained from three calibration curves
[b] 95% confidence limit
[c] Critical values for F at *p* 0.05

3.2. Dissolution of bovine liver samples for the determination of Al and Si

The feasibility of the concomitant determination of Al and Si in the same sample was first tested using nitric acid digestion because this procedure is suitable for the determination of Al in biological tissues. Table 3 shows the results for both elements. Acid digestion is often used for Al determination in animal tissue samples by GFAAS [30-32], and the recoveries were satisfactory for an acid volume of only 3.0 mL. On the other hand, the results in table 3 show that it was not possible to recover the amount of Si added to any of the samples that were acid digested. One factor that may explain this behavior is that Si is unstable in an acidic medium and tends to precipitate [28]. An alternative would be the alkalinization of the samples prior to the Si determination [33]; however, this increases the risk of contamination by adding an additional reagent.

Similar approach was carried out by Schrijver at al [34], for the determination of Al and Si in polyamide samples by GF AAS. Although the same elements were measured, samples are somewhat different. In our case, sample dissolution in an acid milieu resulted in Si precipitation. However, in overall, the results of both studies in terms of method performance are comparable. The the use of matrix-matched standards prepared in formic acid (used for sample dissolution) in their study resulted in an improvement in the accuracy of the GF-AAS Si measurement.

An alternative to acid digestion is pretreatment with TMAH, a strong and water soluble base. Because only a small amount of TMAH is used, this procedure causes a low dilution of the sample resulting in a higher analyte concentration in the solution enhancing the determination of trace elements [19, 20]. However, analysis of the samples solubilized indicated that TMAH greatly reduces the life of the graphite furnace. In this work, it was observed that after 200 heating cycles, the graphite furnace presented roughness and defragmentation on its internal surface. In addition, the absorbance signals widened and often duplicated, leading to incorrect recoveries of the spiked samples. One alternative for reducing this degradation has been to coat the tube with a permanent modifier. Thus, Zr was tested as a permanent modifier, and its use increased the useful life of the tube approximately by a factor of 3 from 200 to over 600 burns. The coating was performed again after 300 burns, when the analyte signal changed, or when the recoveries of Si and Al in the spiked samples were unsatisfactory. Little or no change in the surface of the furnace was observed, confirming the efficacy of the permanent modifier to increase the useful life of the furnace.

The liver samples were completely dissolved after 1 hour in a water bath at 100 °C for all of the tested volumes (0.5 to 2.0 mL) of TMAH, and good recoveries were obtained for both elements with the various TMAH volumes. Thus, a volume of 0.5 mL of TMAH was chosen as it was large enough for sample dissolution and presented the best Al and Si recoveries (Table 3). Moreover, being that is was the smallest volume caused less deterioration of the graphite furnace during the measurements. The option for the measurement of Si using the standard addition technique was chosen because when a standard calibration method was used, the recoveries were very low. Because the calibration curve was obtained under similar conditions to those of the samples, the Si recoveries were significantly improved (Table 3).

Table 3: Recoveries of the Al and Si added to liver samples decomposed by nitric acid or TMAH.

Reagent (mL)	Samples	Aluminum		Silicon	
		µg L^{-1}	% recovery	µg L^{-1}	% recovery
Nitric Acid					
1.0	Blank[1]	30.9		9.0	
	Liver	32.5		35.6	
	Liver+spk[2]	164.5	132.0	35.8	-
2.0	Blank[1]	17.8		N.D.	
	Liver	28.3		N.D.	
	Liver+spk[2]	193.8	165.5	N.D.	-
3.0	Blank[1]	38.4		N.D.	
	Liver	41.6		N.D.	
	Liver+spk[2]	126.1	84.5	N.D.	-
5.0	Blank[1]	52.0		N.D.	
	Liver	118.3		N.D.	
	Liver+spk[2]	176.3	58	N.D.	-
TMAH					
0.5	Blank[1]	126.9		148.7	
	Liver	160.2		544.4	
	Liver+spk[2]	258.1	97.9	835.7	97.1
1.0	Blank[1]	249.8		34.0	
	Liver	298.3		93.6	
	Liver+spk[2]	391.2	92.9	426.3	110.9
2.0	Blank[1]	27.4		53.4	
	Liver	74.6		206.6	
	Liver+spk[2]	187.5	112.9	416.9	70.1

[1] Blank = Nitric acid or TMAH; [2]Liver+spk= addition of 100 µg L^{-1} Al and 300 µg L^{-1} Si
N.D. = not determined.

3.3. Application to certified and real samples

To check the method performance, standard references materials were analyzed. It was not possible to find a SRM containing both elements; therefore. 2 different reference materials were analyzed including SRM 1577b for Al and SRM 1577c for Si. Although the concentration value for Al in SMR 1755b is considered noncertified (without an estimated uncertainty), a concentration of 3 µg g^{-1} is presented in Table 4 of the certificate. The Al and Si concentrations found with the proposed procedure are shown in Table 4 as well as the certificate values. Student's *t*-test was performed on the individual values. The computed *t*-values were 1.14 and 2.74, for Al and Si respectively. These values were lower than tabulated *t*-value of 2.78 ($p<0.05$), indicating no significant difference between the measured and the certified values. For both elements, the measured levels can be considered to be satisfactory.

Table 4: Results of the analysis of bovine liver SRM for Al and Si and the reference values. n = 3.

Sample	Element	Reference	Found
SRM 1577b	Al	3 μg g^{-1}	3.23±0.15 μg g^{-1}
SRM 1577c	Si	6 mg kg^{-1}	6.17±0.18 mg kg^{-1}

This method was then used for the determination of Al and Si in 5 different samples of bovine liver. Bovine liver was chosen as a model sample for the following 2 reasons: the existence of bovine liver reference materials containing Al and Si, and among all animal tissues, liver is the most difficult to decompose. Table 5 shows the contents of Al and Si in these samples as well as the recoveries obtained from spiked samples, which were between 97% and 112%. These results along with the results for the SRMs show that these elements were brought into solution quantitatively by the TMAH digestion method and that the optimized GFAAS measurement method is effective.

Table 5: Al and Si in different samples of bovine liver and the recoveries of spiked samples treated with TMAH.

Sample	Aluminum		Silicon	
	Results (μg g^{-1})	% recovery[1]	Results (μg g^{-1})	% recovery[1]
1	2.58	97.6	1.58	100.1
2	3.90	106.7	1.24	112.6
3	2.49	107.5	4.45	102.5
4	2.86	99.27	4.49	98.26
5	3.08	101.9	2.81	101.9

[1] Treatment = addition of 1 μg of Al and 3 μg of Si to each sample.

4. Concluding remarks

In this study a method for the determination of both Al and Si in biological tissues was developed. Concerning simplicity, dissolution with TMAH is very attractive and proved to be efficient. The use of Si as a modifier for Al eliminates the significant influence of this element upon the atomic absorption signal of Al measured by GFAAS. For the determination of Si, palladium nitrate was the most suitable modifier. Compared with acid digestion, the dissolution of animal tissue (liver) with TMAH showed better recoveries for both elements. Inconveniences related to the use of TMAH were overcome. The decreasing of the graphite furnace life was surpassed by the use of Zr as a permanent modifier, and the high blanks were minimized by using the standard addition method for Si determination. This method can be useful for the concomitant determination of Si and Al in biological samples by GFAAS.

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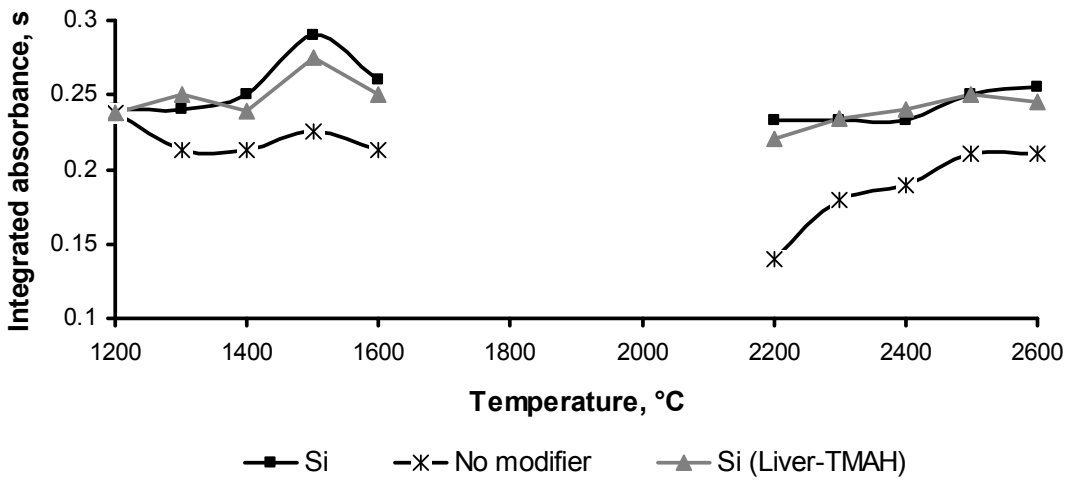


Figure 1: Pyrolysis and atomization curves for 1.0 pg of Al with and without 0.6 µg of Si as a modifier, and the pyrolysis and atomization curves for 1.0 pg of Al in a bovine liver sample solubilized with TMAH with 0.6 µg of Si as a modifier. Zr was used as a permanent modifier in all cases.

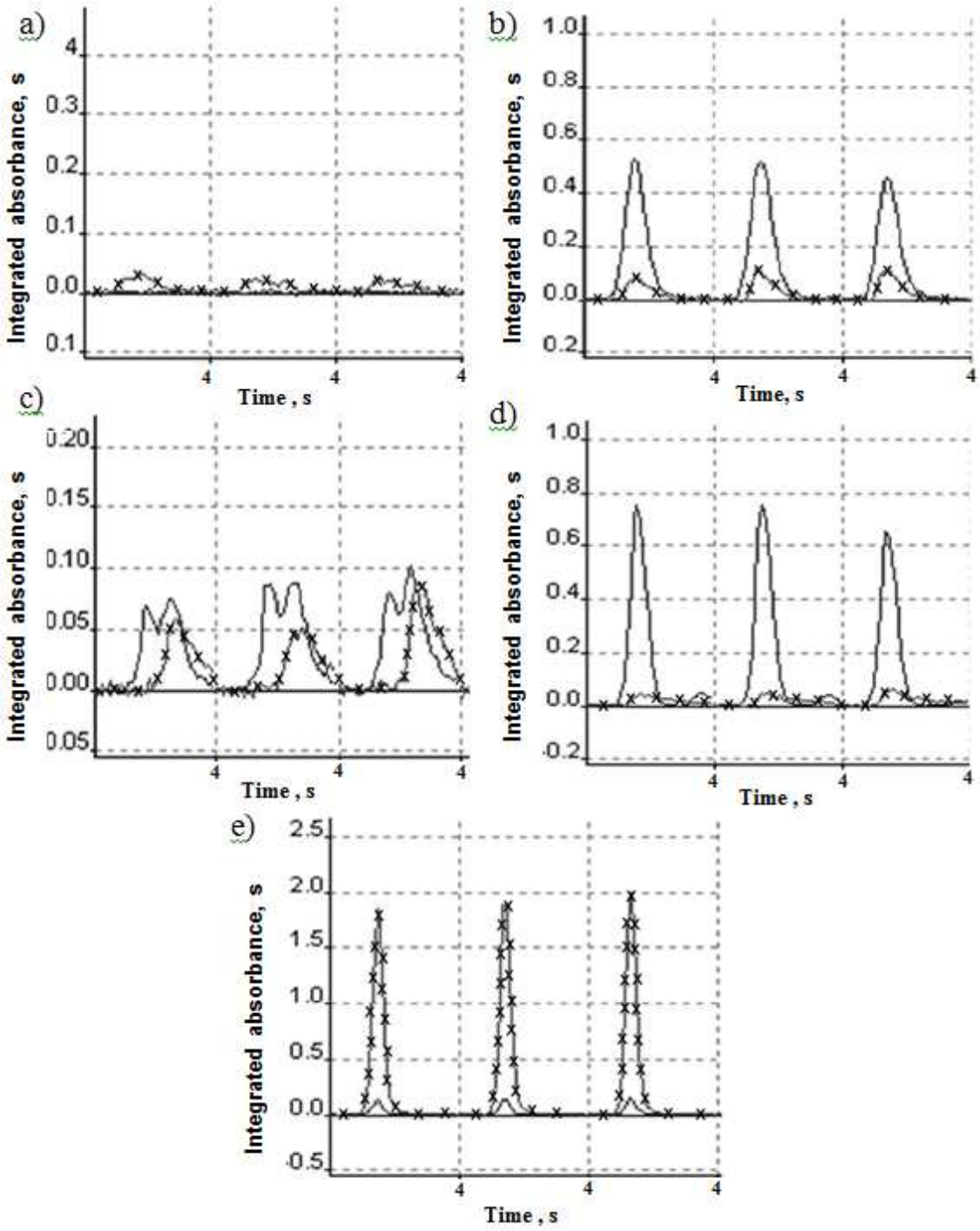


Figure 2: Absorbance signals of 20 pg of Si without modifier (a) and with the following modifiers: 0.5 µg of $CaCl_2$ (b), 6 µg of Pd + 0.2 µg of Ca (c), 6 µg of Pd + 2 µg of Mg (d) and 10 µg of Pd (e) with a pyrolysis temperature of 1200 °C and an atomization temperature of 2600 °C. (——) signal background correction and (—X—) Si signal.

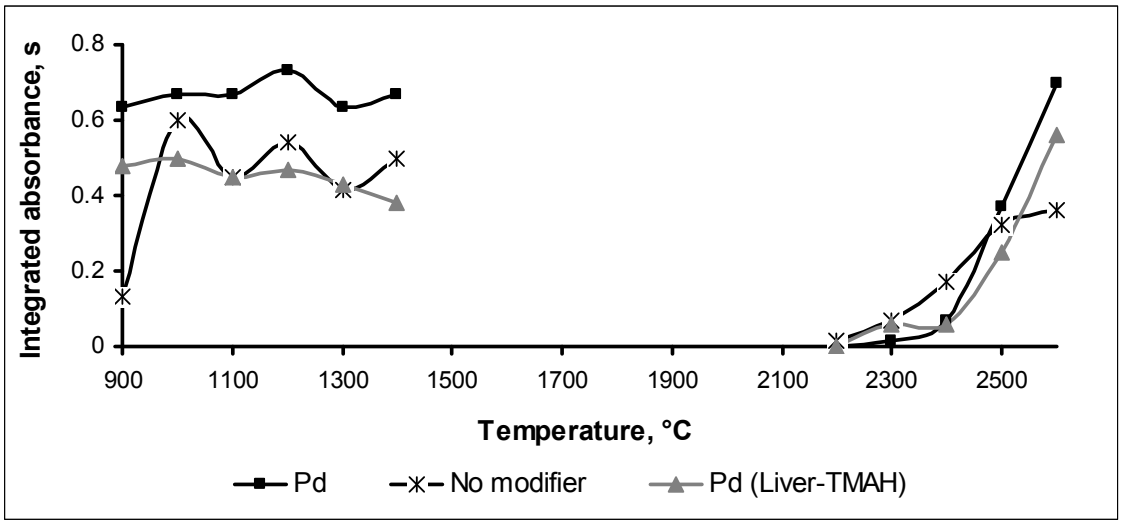
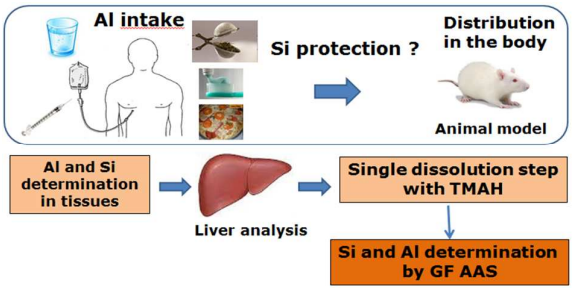


Figure 3: Pyrolysis and atomization curves for 10 pg of Si with and without 10 µg of $Pd(NO_3)_2$ as a modifier, and curves for 10 pg of Si in a bovine liver sample solubilized with TMAH with 10 µg of $Pd(NO_3)_2$ as a modifier. Zr was used as a permanent modifier in all cases.



Liver dissolution with TMAH allowed for the determination of Al and Si in the same sample by GF AAS. The method was validated against standard reference materials.