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The use of electrothermal vaporizer coupled to the inductively coupled plasma mass spectrometry for the determination of arsenic, selenium and transition metals in biological samples treated with formic acid

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ABSTRACT

A fast method for the determination of As, Co, Cu, Fe, Mn, Ni, Se and V in biological samples by ETV-ICP-MS, after a simple sample treatment with formic acid, is proposed. Approximately 75 mg of each sample is mixed with 5 mL of formic acid, kept at 90 °C for 1 h and then diluted with nitric acid aqueous solution to a 5% (v/v) formic acid and 1% (v/v) nitric acid final concentrations. A palladium solution was used as a chemical modifier. The instrumental conditions, such as carrier gas flow rate, RF power, pyrolysis and vaporization temperatures and argon internal flow rate during vaporization were optimized. The formic acid causes a slight decrease of the analytes signal intensities, but does not increase the signal of the mainly polyatomic ions ($^{14}N^{35}Cl^+$, $^{14}N^{12}C^+$, $^{40}Ar^{12}C^+$, $^{13}C^{37}Cl^+$, $^{40}Ar^{36}Ar^+$, $^{40}Ar^{35}Cl^+$, $^{35}Cl^{16}O^+$, $^{40}Ar^{18}O^+$) that affect the analytes signals. The effect of charge transfer reactions, that could increase the ionization efficiency of some elements with high ionization potentials was not observed due to the elimination of most of the organic compounds during the pyrolysis step. External calibration with aqueous standard solutions containing 5% (v/v) formic acid allows the simultaneous determination of all analytes with high accuracy. The detection limits in the samples were between 0.01 (Co) and 850 $\mu g\,kg^{-1}$ (Fe and Se) and the precision expressed by the relative standard deviations (RSD) were between 0.1% (Mn) and 10% (Ni). Accuracy was validated by the analysis of four certified reference biological materials of animal tissues (lobster hepatopancreas, dogfish muscle, oyster tissue and bovine liver). The recommended procedure avoids plasma instability, carbon deposit on the cones and does not require sample digestion.

1. Introduction

Sample preparation is a critical step of an analytical procedure, and despite all recent advances it still requires further improvement to reach the same high standards of the instrumental techniques required for accurate determination of the analytes [1,2]. Biological tissue samples are analyzed for the determination of trace metals predominately after digesting the sample with oxidizing acids, using either a hot-plate or a microwave oven [3,4]. However, alternative methods such as dry ashing [5,6], solvent extraction [7], ultrasound-assisted extraction with acids [8,9] or enzymatic hydrolysis [10] and pressure assisted chelating extractions [11] have been reported.

The use of organic reagents such as primary amines and tretamethylammonium hydroxide (TMAH) has been proposed as an alternative to the decomposition processes [2]. Formic acid has been reported as an effective reagent to solubilize solid biological samples for the determination of trace metals by electrothermal atomization atomic absorption spectrometry (ETAAS), inductively coupled plasma optical emission spectrometry (ICPOES) [4], vapor generation atomic absorption spectrometry or atomic fluorescence spectrometry (VG-AAS or VG-AFS) [12–16] and inductively coupled plasma mass spectrometry (ICP-MS) [16–18]. Recently, dried deposits of biological tissues solubilized with formic acid were analyzed by laser ablation time of flight ICP-MS [19]. Formic acid was also efficiently used for the solubilization of polyamide polymer prior to the determination of Al, Mn and Si by ET AAS and by ICP-MS with nebulization followed by membrane desolvation to minimize the effect of the formic acid on the plasma [20]. It is less time consuming and easier than the conventional acid digestion, as the solubilization can be performed in the same plastic bottle where the sample was weighed and stored. Among the advantages of the use of the formic acid is the low cost, easiness of handling,

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possible purification to a high degree and a small sample aliquot of just a few milligrams of the sample is required.

The majority of analyses by ICP-MS are carried out on solutions using a conventional pneumatic nebulizer. However, the type of analytical tasks that can be solved by ICP-MS can be extended using a number of other sample introduction techniques that can be easily adapted to ICP-MS. Electrothermal vaporization (ETV) is one of the sample introduction techniques, that is employed in ICP-MS. This alternative technique to solution nebulization presents several advantages [21]. Samples treated with organic reagents, are especially adequate for the determination by ETV-ICP-MS [20–23] since this technique is very robust, not requiring totally dissolved or digested samples. The resulting sample solution can be accurately analyzed, since the organic matter and other matrix components can be volatilized during the pyrolysis step of the furnace temperature program, not interfering with the plasma stability or with the analyte determination. The ETV-ICP-MS has been extended to the micro volume analysis, especially when coupled to a separation/preconcentration technique, as liquid-phase micro-extraction, stir bar sorptive extraction, solid-phase micro-extraction, or capillary electrophoresis [23]. The main ETV applications are the analysis of solids, slurries and complex matrixes, as commented on a recent review by Resano et al. [21].

This study deals with the preparation of biological samples with formic acid for the simultaneous determination of As, Co, Cu, Fe, Mn, Ni, Se and V by ETV-ICP-MS. The chosen analytes include volatile ones as As and Se and less volatile analytes, as the mentioned transition metals. The influences of instrument operating conditions were evaluated. Optimization of sample preparation conditions and of the instrumental parameters concerning the alleviation of spectral and non spectral interferences will be carried out. Calibration against aqueous standards with or without formic acid will be tested. Accuracy will be validated by the analysis of certified reference samples or by comparison with the results obtained by conventional ICP-MS, after digestion. To the best of our knowledge, this approach was never used before for the analysis of biological samples.

2. Experimental

2.1. Instrumentation

An inductively coupled plasma mass spectrometer Elan 6000 equipped with an HGA-600 MS electrothermal vaporizer and an autosampler model AS-60, both from *Perkin-Elmer SCIEX* (Thornhill, Canada) was used. Pyrolytic graphite coated tubes (Part Number BO 091509) from Perkin-Elmer (Norwalk, USA) were used throughout the work. The argon gas with minimum purity of 99.996% was supplied by *White Martins* (São Paulo, Brazil). The experimental conditions are given in Table 1. When the modifier solution was used, it was pipetted first and then the sample solution or the standard solution, using an automatic dispenser. The pipetted volume was always 20 μL.

For comparison purpose, the samples were also digested in a microwave oven Ethos Plus from *Milestone* (Sorisole, Italy), using a four steps program (2:30 min up to 90 °C; 5 min up to 135 °C; 3:30 min up to 190 °C and kept by 10 min at 190 °C). The digested samples were analyzed by ICP-MS using the conventional nebulizer system (cross flow and Scott spray chamber) and external calibration with Rh as internal standard.

2.2. Reagents and samples

The used water was distilled and de-ionized, with a resistivity of 18 MΩ cm, produced by a Milli-Qplus system from *Millipore* (Beadford, USA). Hydrogen peroxide (40%, v/v) from *Vetec* (Rio de Janeiro, Brazil) was used. Concentrated nitric acid (65%, v/v) and hydrochloric acid (37%, v/v) from *Merck* (Darmstadt, Germany) and formic acid (85%, v/v) (*Vetec*) were both purified by sub-boiling distillation in a quartz still from *Kürner Analysentechnik* (Rosenheim, Germany). A Multi-element Standard Solution 3 from *Perkin-Elmer*, Rh mono-elemental standard solution (*Perkin-Elmer*) and Fe and Se mono-elemental solutions from *Merck* were used. A solution of palladium nitrate, $Pd(NO_3)_2$, 10 g L^{-1} from *Merck* was used as chemical modifier/carrier. The analyzed samples were: bovine liver 1577b and oyster tissue 1566a from *National Institute of Standard and Technology*, *NIST* (Gaithersburg, USA); TORT 2 (Lobster Hepatopancreas) and DOLT 3 (Dogfish liver) from *National Research Council Canada*, *NRCC* (Ottawa, Canada).

2.3. Procedures

2.3.1. Sample preparation

Using an analytical balance AG204 from *Mettler Toledo* (Giessen, Germany), approximately 75 mg of each sample were weighed in 15 mL polypropylene flask, 5 mL of the purified formic acid were then added and the mixture was shaken vigorously in order to avoid sticking of the sample power on the flask inner surface. In the sequence, the flask with formic acid and sample was kept at 90 °C in a water bath, during 1 h and the volume was completed to 10 mL. Prior to the analysis, another dilution was carried out, being 1 mL diluted to 10 mL with water and nitric acid to a final concentration of 1% (v/v) (the final concentration of formic acid was 5% (v/v)) to reduce the signal obtained for Fe and Cu and work in the linear range of the calibration curves for these elements. The sample solution was analyzed using ETV-ICP-MS. This sample treatment procedure was based on a previous work of Tormen et al. [17].

Microwave assisted digestion of the sample to check the concentrations of non certified elements were carried out. For the digestion procedure, about 150 mg of each sample were mixed with 5 mL of HNO_3, 2 mL of H_2O_2 and 2 mL of water in a PTFE flask that was submitted to the temperature program and diluted to 30 mL. Before the analysis the solutions were diluted, 2–10 mL with water, Rh was added to a final concentration of 5 mg L^{-1}. The solutions were analyzed by ICP-MS, using the conventional sample introduction system.

2.3.2. Effect of formic acid

For the study of the effect of formic acid on the analyte intensity signal, solutions were prepared with 1% (v/v) nitric acid, different concentrations of formic acid (0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80 and 90%, v/v) and enriched with aqueous analytes standards to a final concentration of 10 μg L^{-1}. A blank solution was always measured and taken into consideration. For the investigation of the polyatomic ions that may result in spectral interference, solutions with increasing formic acid concentrations (same as above) and with 0.1% (v/v) of HCl and 1% (v/v) HNO_3, were measured. The optimized and adopted conditions were 1300 W RF power and 1.0 L min^{-1} gas flow rate, internal gas flow rate of 150 mL min^{-1}, pyrolysis and vaporization temperature of 1000 and 2400 °C, respectively, and 2 μg of Pd.

2.3.3. Analytes determination

For the analysis of the sample treated with formic acid, the optimized and adopted conditions previously mentioned, were used. External calibration was carried out with aqueous standards in 1% (v/v) nitric acid, containing or not 5% (v/v) formic. The analytes concentrations were 0.5, 1, 2.5, 5, 10, 25 and 50 μg L^{-1}. The analysis of the digested samples were carried out using a conventional introduction system, RF power of 1100 W and nebulizer gas flow rate of 1.025 L min^{-1}.

Table 1
(A) ICP-MS operating and data acquisition parameters and (B) ETV temperature program.

(A) ICP-M operating and data acquisition parameters

RF power (W)	1300
Carrier Ar flow rate (L min^{-1})	1.0
Dwell time (ms)	25
Scan mode	Peak-hopping
Sweeps/reading	1
Readings/replicate	60
Replicates	3
Signal measurement mode	Integrated
Integration time (s)	16.8
Sampler and skimmer	Pt

(B) ETV temperature program (20 μL of the modifier and 20 μL of the sample solutions)

Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Internal flow rate (mL min^{-1})
Cleaning	2700	1	5	300
Cooling	20	1	5	300
Drying	90	5	10	300
Drying	120	5	15	300
Pyrolysis	1000	10	15	300
Cooling	20	1	3	150
Vaporization	2400	1	15	150
Cooling	20	1	5	300

3. Results and discussion

3.1. Instrument optimization

Due to the large differences on the thermal properties of the studied analytes, the optimization of the pyrolysis and vaporization temperatures were carried out for all analytes. Only the curves for two isotopes of elements of very different volatilities, $^{75}As^+$ and $^{55}Mn^+$, are shown in Fig. 1. The pyrolysis curves for all studied elements decreases with temperature. In general, the decrease is very pronounced for the region of lower temperatures, from 200 °C up to 400–500 °C. For intermediate pyrolysis temperatures, the intensity decreases, but less intensively and finally the curves decrease very steeply for temperatures higher than the optimum pyrolysis temperatures, as expected. The curves for $^{82}Se^+$ are similar to the shown curves for $^{75}As^+$, while the curves for the other studied transition metals are similar to the curves for $^{55}Mn^+$. For $^{82}Se^+$ and also for $^{75}As^+$, the signal intensity for the enriched sample, without modifier/carrier, decreases very pronouncedly already at very low temperatures as the amount of carbon introduced into the plasma and, consequently, its aerosol carrier effect also decreases, as it will be discussed below. For the aqueous standard, also without Pd, the steepest decrease occurs at higher pyrolysis temperature. It is known that the presence of carbon in the plasma may enhance the analyte ionization, especially of some elements of high ionization potentials, such as $^{75}As^+$ and $^{82}Se^+$, possibly due to charge transfer reactions from the carbon to the analyte [17,24] and may also act as a carrier for the aerosol produced in the ETV. In the presence of Pd, the stabilization for the analyte either in the standard solution or in the treated sample, is remarkable. The amount of carbon in the plasma varies with the pyrolysis and vaporization temperatures, as shown for $^{13}C^+$ inFig. 1. When the pyrolysis temperature increases, the population of $^{13}C^+$ in the plasma decreases for pyrolysis temperature up to around 650 °C, consequently the ionization efficiency for some elements with a high ionization potential, such as As and Se, also must decrease. In addition, the carrier effect of carbon for all analytes should also decrease in this pyrolysis temperature range. For higher pyrolysis temperatures, from 650 °C to the highest studied temperature of 1650 °C, the signal intensity at m/z of $^{13}C^+$ is quite stable as it is produced mainly by volatilization of the carbon from the graphite tube at a constant vaporization temperature, considering the elimination of the matrix. The signal intensity of $^{13}C^+$ increases as the vaporization temperature increases, as shown in Fig. 1, meaning that the carbon effect as carrier and as ionization enhancer, for some elements, also should increase. However this effect was observed only for the curves without palladium. When palladium was present, by increasing the vaporization temperature, the signal intensity decreases, as the signal shape becomes narrower and better defined. It is interesting to note that the presence of Pd, reduces the population of $^{13}C^+$ in the plasma, for the aqueous standard solution without any organic compound and for the sample treated with formic acid, indicating interaction between carbon of the graphite tube with the modifier, as suggested by Ortner et al. [25] to explain the Pd action in GF AAS, as chemical modifier. As a relatively high pyrolysis temperature was adopted, 1000 °C, the discussed possibilities at very low pyrolysis temperature do not affect the sample analysis. For $^{55}Mn^+$, as shown in Fig. 1, and for the other studied transition elements, the effect of the modifier is much less pronounced, as it was expected. For these more stable analytes, the ionization enhancement due to carbon charge transfer reactions must be negligible, as their ionization potential is relatively low and their ionization efficiency is close to 100%. However, carbon may act as carrier for the sample aerosol, as already mentioned. The adopted compromise conditions were pyrolysis at 1000 °C and vaporization at 2400 °C.

The effect of the Pd mass was studied for all analytes, but for convenience, only the curves for $^{75}As^+$ and $^{55}Mn^+$ are shown in Fig. 2. The optimization was carried out for the aqueous inorganic standard and for the sample treated with formic acid. For aqueous inorganic standard, due the absence of matrix that could act as a carrier and due the thermal stabilization the use of palladium increases the signal of all elements, especially for $^{75}As^+$ and $^{82}Se^+$. However, for the treated sample, the carrier effect of Pd is less clear, because the sample matrix and the formic acid may act as carrier for the sample aerosol. For high mass of Pd, 8 μg, the signal decreases up to 40% possibly due to space charge effect [26]. In contrast, for example, 2 μg of Pd is enough to increase 4 times the transport efficiency for $^{75}As^+$ and $^{82}Se^+$. For the less volatile elements, the increase in the transport efficiency due to Pd is less important, especially for the sample, as explained above. A mass of 2 μg of Pd produces the best signals and was adopted.

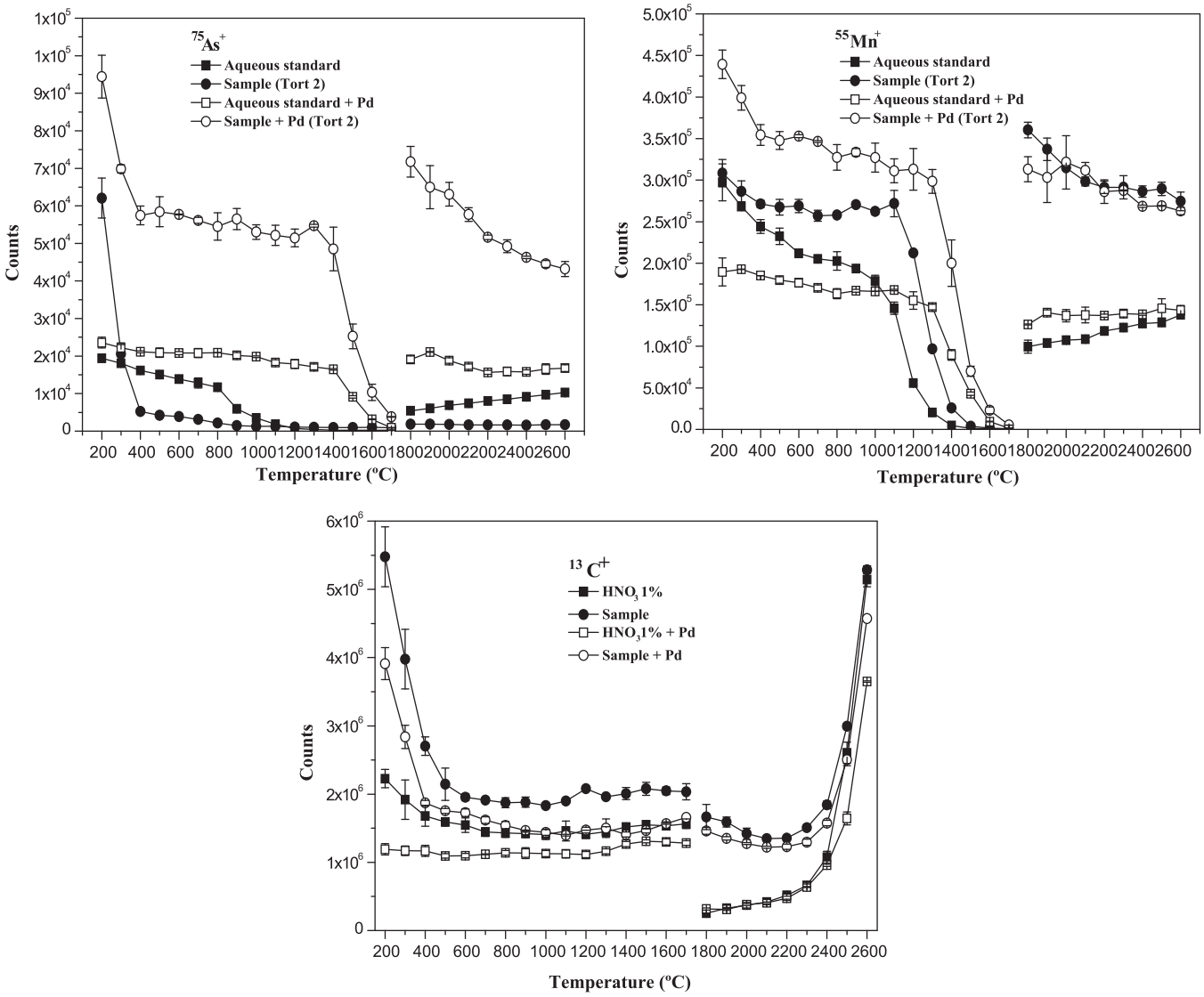

Fig. 1. Pyrolysis and vaporization curves for $^{75}As^+$, $^{55}Mn^+$ and $^{13}C^+$. Aqueous standard solution: with (□) and without (■) Pd, as carrier/modifier; sample solution in formic acid: with (○) and without (●) Pd. Analyte concentration: 10 μg L^{-1} in 1% (v/v) HNO_3 for aqueous standard solution and sample TORT 2 treated with formic acid and enriched with 5 μg L^{-1} of Se. The mass of Pd was 2 μg (20 μL of a 100 μg mL^{-1} Pd solution). Sample volume: 20 μL. Vaporization at 2500 °C for the pyrolysis curves; pyrolysis at 600 °C for the vaporization curves. Internal gas flow rate of 150 mL min^{-1}, carrier gas flow rate of 1.025 L min^{-1} and RF power of 1100 W.

All other instrumental parameters, RF power, carrier gas flow rate and ETV inner gas flow rate, were optimized for the highest signal intensity in an univariate way and are listed in Table 1. By increasing the RF power from 800 W (cold plasma) to 1400 W, the relative signal intensity, for the treated sample, increases up to 3 times, depending on the analyte. A more energetic plasma than that normally used for aqueous solutions was required due to the presence of formic acid. A RF power of 1300 W was adopted, which is higher than that normally used in our instrument due to the presence of formic acid.

3.2. Effect of the formic acid content

The effect of the formic acid content on the analyte signal intensity was evaluated increasing the formic acid percentage in the standard solution, as shown in Fig. 3(a). The highest signal intensity was obtained for the solution without formic acid, however, the decrease in the signal intensity is not very pronounced, even for high formic acid content, oscillating between the maximum and 80% of the maximum. The real intensities (counts) obtained for the solution without formic acid were 41,413 ($^{51}V^+$), 169,573 ($^{55}Mn^+$), 4427 ($^{57}Fe^+$), 80,357 ($^{59}Co^+$), 11,185 ($^{60}Ni^+$), 35,088 ($^{63}Cu^+$), 34,942 ($^{75}As^+$), 6528 ($^{82}Se^+$) and 2,203,608 counts for $^{13}C^+$. The introduction of formic acid [17] or others organic compounds, as acetone and methanol [27], ethanol [24] and acetic acid [28] in comparison to the introduction of aqueous solutions, using conventional nebulization systems, resulted in a signal intensity improvement for the more difficult to ionize elements, such as $^{75}As^+$ and $^{82}Se^+$ and also due to the usually higher nebulization and transport efficiencies of the organic medium. Using an ETV as the sample introduction system, there is no nebulization, and consequently, the physical properties of the solution are less important (however, the penetration of the solution on the graphite layers of the tube depends on the properties). In addition, the solvent and the sample matrix are eliminated in the drying and pyrolysis steps of the ETV temperature program. The monitoring of $^{13}C^+$, for which the signal intensity remains approximately constant, as shown in Fig. 3(a), demonstrates an excellent elimination of the solvent and of the organic matrix decreasing, consequently, the amount of carbon introduced into the plasma, so that the charge transfer ionization

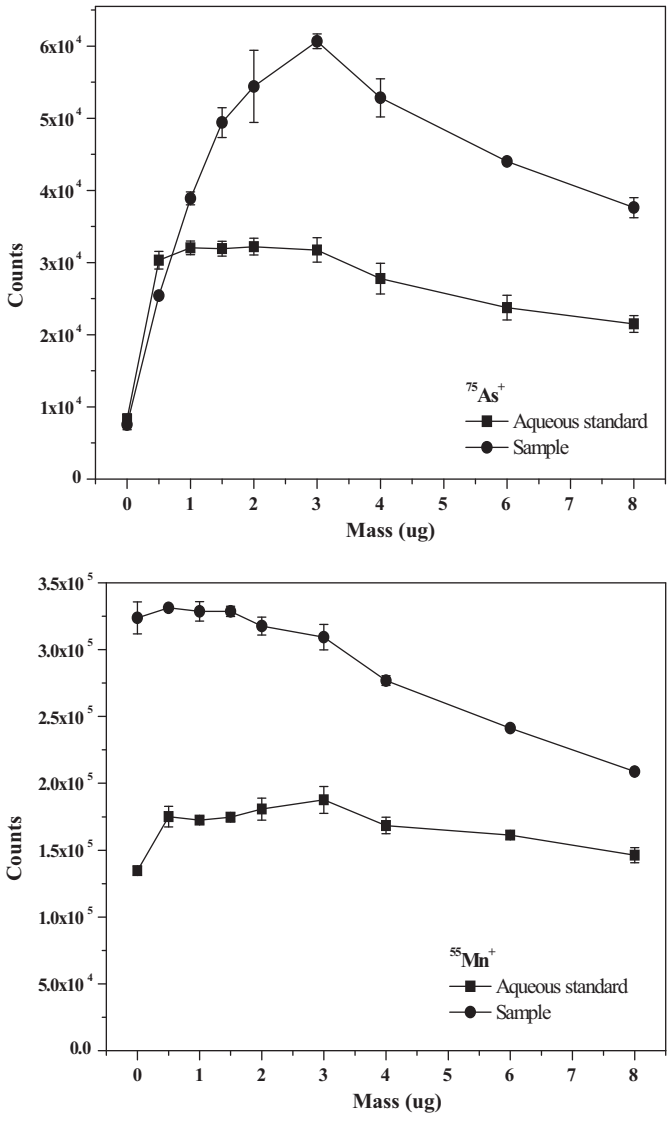

Fig. 2. Palladium mass (μg) optimization for $^{75}As^+$ and $^{55}Mn^+$ in the aqueous standard solution (■) and the sample solution in formic acid (●). Analyte concentration: the 10 μg L^{-1} in 1% (v/v) HNO_3 for aqueous standard solution and sample TORT 2 treated with formic acid and enriched with 5 μg L^{-1} of Se. Modifier volume: 20 μL of Pd solutions; sample volume: 20 μL. Carrier gas flow rate of 1.025 L min^{-1}, RF power of 1100 W, pyrolysis at 1000 °C, vaporization at 2400 °C and internal gas flow rate of 150 mL min^{-1}.

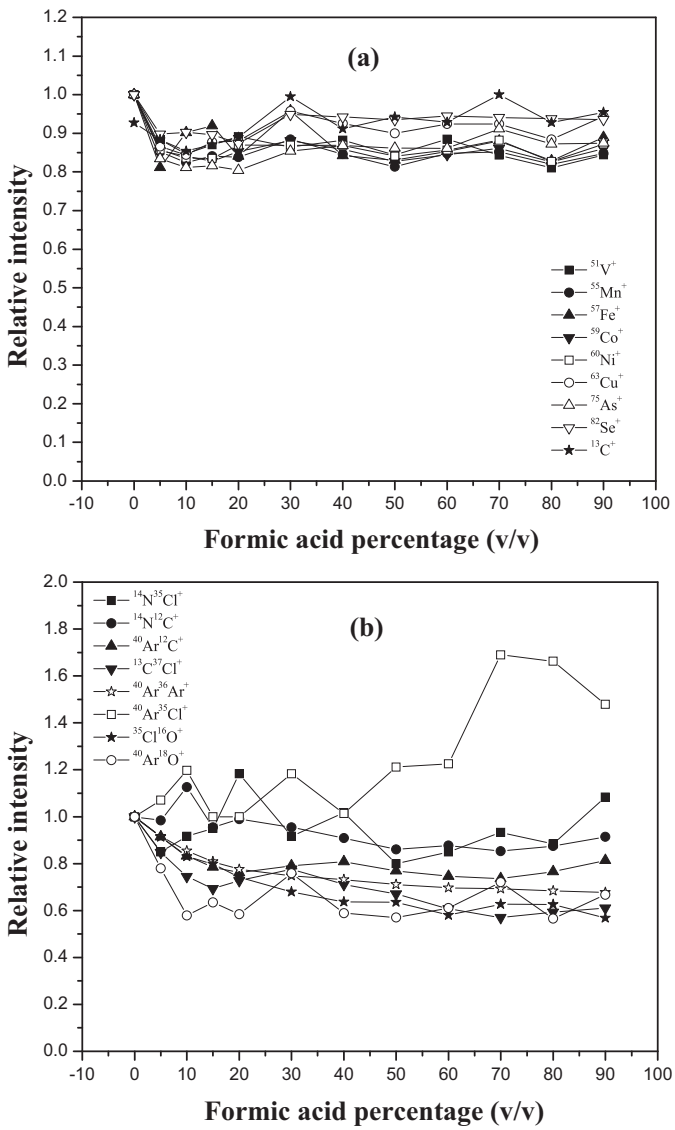

Fig. 3. Effect of formic acid content on the relative signal intensity of the analytes (a) and of typical polyatomic ions (b). Analyte concentrations: 10 μg L^{-1} for the evaluation of the analyte signal. The signal intensity was related to the value for the solution without formic acid. RF power: 1300 W; carrier gas flow rate: 1.0 L min^{-1}; internal gas flow rate: 150 mL min^{-1}, pyrolysis at 1000 °C; vaporization at 2400 °C and 2 μg of Pd as carrier/modifier (20 μL of a 100 μg mL^{-1} Pd solution); sample volume: 20 μL.

from the carbon should be not significant. Carbon volatilized from the graphite tube can also induce ionization of some of the high ionization potential elements, as already discussed; however, its content in the plasma must be approximately constant for a same vaporization temperature.

A study of some important polyatomic ions that could cause spectral interference on some isotopes (not necessarily on the analytical isotopes used in this work), is shown in Fig. 3(b). The signal intensity remain practically constant in relation to the aqueous solution for the studied ion, except for the ion $^{40}Ar^{35}Cl^+$ that shows a slight increase in the signal intensity, which is small in comparison to the increase observed when organic solutions are introduced by nebulization [17]. The real intensities (counts) obtained for the solution without formic acid were 60 ($^{14}N^{35}Cl^+$), 4486 ($^{14}N^{12}C^+$), 138,752 ($^{40}Ar^{12}C^+$), 349 ($^{13}C^{37}Cl^+$), 3846 ($^{36}Ar^{40}Ar^+$), 71 ($^{40}Ar^{35}Cl^+$), 857 ($^{35}Cl^{16}O^+$) and 544 counts for $^{40}Ar^{18}O^+$. Although the sample solution has a high amount of organic compounds and salts, the furnace temperature program promotes the elimination of most of the solvent and matrix, reducing the possibility of interference and allowing the calibration with aqueous inorganic calibration. Certainly, this possibility is an important advantage of ETV in relation to other sample introduction systems for ICP-MS, making this technique adequate for different and complex samples.

3.3. Analytical application

The formic acid treatment of dried and milled biological samples should be carried out under heating, in order to accelerate the sample dissolution. In our proposed procedure, the solubilization time could be decreased to 1 h by increasing the temperature to 90 °C. Solid particles may remain in the sample solution if the solubilization is carried out at temperatures lower than 80 °C. If the sample solution is too viscous, the solubilization may take several hours. Tissues with cellulose and lignins from botanical samples

Table 2
Figures of merit for the trace elements determination in biological samples treated with formic acid using an ETV as introduction sample system (proposed method). $n = 10$ (LOD) and $n = 3$ (RSD).

Analyte	Isotope (u.m.a.)	Proposed method (ETV as sample introduction system)				Previous work [17] (micronebulizer as sample introduction system)	
		Aqueous calibration without formic acid		Aqueous calibration with formic acid 5% (v/v)		Aqueous calibration with formic acid 50% (v/v)	
		LOD ($\mu g\,kg^{-1}$)	RSD (%)	LOD ($\mu g\,kg^{-1}$)	RSD (%)	LOD ($\mu g\,kg^{-1}$)	RSD (%)
V	51	10	1.5	5.0	1.6	4.5	1.0
Mn	55	5	0.1	0.5	1.8	20	0.3
Fe	57	100	1.8	850	1.6	710	0.7
Co	59	0.010	1.1	1.0	1.9	3.0	2.1
Ni	60	15	10	25	4.8	8.0	1.3
Cu	63	25	1.6	5.0	1.5	10	1.0
As	75	10	0.3	0.5	1.0	90	1.7
Se	82	100	0.8	10	8.4	100	4.2

The detection limit (LOD) was defined as 3 times the standard deviation of ten measurements of the blank concentration multiplied by the sample dilution factor.

are not completed solubilized [17] and the resulting mixture is a slurry that can be injected in the graphite furnace. The amount of formic acid is an important factor, as volumes smaller than 2.5 mL, for 75 mg of the dried sample, may result in suspended matter or in a very viscous solution that is difficult to be pipetted. By using more concentrated formic acid, 99% (v/v) instead of 85% (v/v), for example, solubilization is improved.

The analysis of the treated samples was carried out using two calibration strategies, aqueous inorganic standard solutions without formic acid and with 5% (v/v) of formic acid (same amount of formic acid in the samples after the dilution). In Table 2 are shown the merit figures for the two calibrations strategies. For both strategies, the detection limits were low enough to detect and quantify the analytes. The detection limit (LOD) was defined as 3 times the standard deviation of 10 measurements of the blank and was expressed in relation to the dried sample mass. For most of the elements, the RSD were below 2%, which is excellent taking into account the type of sample introduction system. Comparing with the merit figures obtained in a previous work [17] that used sample treatment with formic acid and a micronebulizer as sample introduction system, they are similar, although the dilution of the sample in this work is 10 times higher in order to have all analytes, including Cu and Fe, in linear range. The high detection capacity of ETV-ICP-MS certainly is another important advantage of this technique. For lower dilutions or no dilution, the determinations of Fe and Cu would be hampered, as the non-linear part of the curves would be used; however, the determination of the other minor and trace elements could also be easily accomplished. For lower dilutions or no dilution, lowers LOD values can be expected in comparison to the ones obtained after 10-times dilution, especially because of the elimination of the sample matrix before vaporization, independently of the dilution. As shown in Fig. 3, even at high concentrations of formic acid, the analytical signal remains constant, indicating that the pyrolysis temperature is high enough to eliminate the sample matrix. Most probably, the lowest detection limits would be attainable with no dilution, around 10 times better than the ones shown in Table 2, but they were not experimentally determined.

For the analytes quantification, external calibrations without formic acid or external calibration with 5% (v/v) formic acid, were tested for four certified reference materials. The results, expressed as the average ± confidence interval at 95%, are shown in Table 3. The analyte concentrations for non certified elements were obtained by the analysis of the acid digested samples in a

Table 3
Obtained concentrations, in $mg\,kg^{-1}$, in biological certified reference materials (average ± confidence interval 95%). The samples were treated with formic acid using calibration against aqueous standard solutions, without and with 5% (v/v) formic acid. $n = 3$.

	TORT 2			Bovine Liver 1577b		
	Certified	Without formic acid	With formic acid	Certified	Without formic acid	With formic acid
V	1.64 ± 0.19	1.73 ± 0.28	1.70 ± 0.04	0.123	0.11 ± 0.03	0.14 ± 0.04
Mn	13.6 ± 1.2	12.6 ± 3.6	16.1 ± 2.3	10.5 ± 1.7	9.01 ± 1.82	12.30 ± 1.10
Fe	105 ± 13	85 ± 12	110 ± 20	184 ± 15	150 ± 15	210 ± 20
Co	0.50 ± 0.09	0.429 ± 0.07	0.606 ± 0.080	0.250	0.192 ± 0.031	0.271 ± 0.024
Ni	2.50 ± 0.20	1.97 ± 0.22	2.50 ± 0.04	0.27 ± 0.03[a]	0.29 ± 0.03	0.27 ± 0.05
Cu	106 ± 10	98.2 ± 15.2	110 ± 25	160 ± 8	128 ± 3	170 ± 6
As	21.6 ± 1.8	24.5 ± 3.7	22.3 ± 4.8	0.050	0.042 ± 0.016	0.040 ± 0.016
Se	5.63 ± 0.67	5.0 ± 0.1	5.8 ± 0.1	0.730 ± 0.060	0.6 ± 0.1	0.7 ± 0.1
	Oyster tissue 1566a			DOLT 3		
	Certified	Without formic acid	With formic acid	Certified	Without formic acid	With formic acid
V	4.70 ± 0.20	4.71 ± 0.36	4.80 ± 0.60	0.67 ± 0.03[a]	0.57 ± 0.10	0.62 ± 0.11
Mn	12.3 ± 1.5	9.31 ± 0.65	12.50 ± 0.60	10.1 ± 2.1[a]	8.70 ± 1.05	11.2 ± 1.6
Fe	540 ± 15	320 ± 27	430 ± 40	1480 ± 60	1190 ± 10	1600 ± 10
Co	0.570 ± 0.110	0.216 ± 0.035	0.261 ± 0.018	0.290 ± 0.040[a]	0.222 ± 0.023	0.296 ± 0.030
Ni	2.25 ± 0.45	2.10 ± 0.45	1.90 ± 0.20	2.72 ± 0.35	2.14 ± 0.12	2.34 ± 0.24
Cu	66.3 ± 4.3	50.6 ± 2.2	63.3 ± 3.5	31.2 ± 1.0	28.3 ± 3.3	33.3 ± 4.6
As	14.0 ± 1.2	12.7 ± 3.1	12.6 ± 0.2	10.2 ± 0.5	10.3 ± 2.2	11.4 ± 2.4
Se	2.20 ± 0.25	1.7 ± 0.2	2.2 ± 0.2	7.06 ± 0.48	5.5 ± 0.7	6.5 ± 1.1

[a] Values obtained after microwave assisted sample digestion and determination using a conventional introduction sample system.

microwave system, using a conventional cross flow nebulizer and a double pass Scott chamber are also shown in Table 3. Accuracy was the main criterion for selecting the best conditions for sample analysis. Comparing the obtained results with the certified values or with the values obtained after sample digestion, both calibrations can be used for the determination of all analytes. However, the use of calibration in the presence of formic acid produced a slightly better concordance with the certified values. The obtained concentrations with solutions containing formic acid were slightly higher than those obtained for calibration with aqueous solutions without formic acid. As high ionization potential elements, such as As and Se were included as analytes, the good accuracy obtained using calibration either without or with formic acid in the standard solutions demonstrate once more that the charge transfer ionization from the carbon is not significant, since the ETV removes most of the solvent and sample matrix.

4. Conclusions

Using the ETV, it is possible to introduce solutions with high percentage of formic acid in order to determine several trace elements in biological samples treated with formic acid. Formic acid effect on the analyte sensitivity, possibly due charge transfer ionization, was observed when low pyrolysis temperature was applied. However, using the optimized conditions, the introduction of solutions with up to 90% (v/v) of formic acid without interference, is possible. The use of Pd as modifier/carrier is recommended mainly to equalize the analyte transport to the plasma for the standard and sample solutions. The analysis of certified reference materials demonstrates good accuracy for all elements, using calibration solutions with or without formic acid. Low detection limits and high precision were obtained. The recommended procedure for sample treatment is easy, rapid, less expensive and environmental friendly when compared to acid digestion, for example, allowing a high sample throughput, without leaving dangerous waste. In addition, the proposed analytical method avoids plasma instability, carbon deposit on the cones and does not require sample digestion. The obtained data demonstrate once again the robustness of the ETV for sample introduction in ICP-MS, as the solvent and concomitants are mostly eliminated during the temperature program.

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