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Determination of trace elements in biological samples treated with formic acid by inductively coupled plasma mass spectrometry using a microconcentric nebulizer

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ABSTRACT

A simple and fast method for the determination of As, Ba, Cd, Co, Cu, Fe, Ga, Mn, Mo, Ni, Pb, Rb, Se, Sr, Tl, U, V and Zn in biological samples by inductively coupled plasma mass spectrometry (ICP-MS), after sample solubilization with formic acid and introduction by a microconcentric nebulizer, is proposed. The sample is mixed with formic acid, kept at 90 °C for one hour and then diluted with nitric acid aqueous solution to a 50% v/v formic acid and 1% v/v nitric acid final concentrations. The final sample solution flow rate for introduction into the plasma was 30 μL min^{-1}. The optimized and adopted nebulizer gas flow rate was 0.7 L min^{-1} and RF power was 800 W. These conditions are very different than those normally used when a conventional nebulizer is employed. Rodhium was used as internal standard. External calibration against aqueous standard solutions, without formic acid, could be used for quantification, except for As, Se and Zn. However, external calibration with 50% formic acid allows the determination of all analytes with high accuracy and it is recommended. The detection limits were between 0.0005 (Tl) and 0.22 mg kg^{-1} (Fe) and the precision expressed by the relative standard deviations (RSD) were between 0.2% (Sr) and 3.5% (Ga). Accuracy was validated by the analysis of four certified reference biological materials of animal tissues, comparing the results by linear regressions and by the t-test at a 95% confidence level. The recommended procedure avoids plasma instability and carbon deposit on the cones.

1. Introduction

The sample preparation obviously is one of the most important steps for trace elements determination; therefore success in this step is required for good results. Among the most used methodologies for biological samples treatment is the wet decomposition, however, it is time consuming, can require high amounts of corrosive and toxic reagents that increase the cost of the analyses and can cause sample contamination. Besides, the high temperature frequently reached during the procedures can cause losses of the most volatile elements [1]. As an alternative to the decomposition process, the solubilization with organic reagents, such as primary amines and tetramethylammonium hydroxide (TMAH), have been proposed [2]. This treatment was applied with success to the determination of many elements in several samples by electrothermal atomic absorption spectrometry (ET AAS) [3–5], inductively coupled plasma optical emission spectrometry (ICP-OES) [6] and inductively coupled plasma mass spectrometry with electrothermal vaporization (ETV-ICP-MS) [7]. Among the main advantages of this solubilization strategy is the low cost and the fastness of the process, however, these reagents have unpleasant odor and are difficult to be found with high purity.

Recently, the use of the formic acid as alternative to TMAH was suggested, in this sense, Scriver et al. [8] proposed the use of this reagent for the solubilization of marine tissues for the determination of Ag, As, Cd, Cu, Cr, Fe, Ni, and Se by ET AAS using external calibration, obtaining good results. More abundant elements, such as Na, Ca, K and Mg were also determined by ICP-OES, however the analyte addition calibration had to be employed for sample matrix effects correction. Kan et. al. [9], also proposed formic acid for the solubilization of biological tissues concerning the determination of total Hg by cold vapor atomic absorption spectrometry (CV AAS), but the most precise results were obtained using calibration by the analyte addition technique. The procedure could also be used in speciation analysis. Among the advantages observed in the use of the formic acid are the low cost, easiness of handling, possible purification to a high degree and the possibility of using just few milligrams of the sample. In addition, there are several other formic acid applications in the determination of trace elements. For example, formic acid was used for the photochemical vapor generation of Se by atomic fluorescence spectrometry (AFS) and by ICP-MS, without a chromatographic separation [10] and of Se, As, Sb, Bi, Te, Sn, Pb, Hg and Cd by AAS and AFS [11–13]. Formic acid was also used in liquid extractions of metal species in mussel tissue and in marine sediment [14] and of As species in plants [15,16]. In case of liquid

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extractions, the elements are determined in the formic acid medium, after extraction.

The introduction of samples treated by wet digestion in ICP-MS using conventional nebulizers is usually simple, since the measuring solution is frequently an aqueous acid solution. However, usually it is not possible to introduce directly by conventional pneumatic nebulizers samples treated with organic compounds, since the organic load may extinguish the plasma and may form carbon deposits on the cones and lenses. It also can cause severe non-spectral interferences, requiring dilution or matrix matching calibration. Spectral interference by carbon polyatomic ions is also a problem [17–19]. Some special sample introduction systems were used to analyze organic samples, such as electrothermal vaporization (ETV) [7,20–22], ultrasonic nebulization coupled to the desolvation [23–25] and the use of oxygen as auxiliary gas [20,25,26]. Micronebulizers, capable of generating a fine and stable aerosol at flows as low as 1 μL min^{-1}, have also been used. They are usually stable and precise, possessing high tolerance to dissolved solids. Nowadays, several of the applications of the micronebulizers are on the coupling of separation methods to the ICP-MS for speciation procedures, because of the small solution volumes obtained after the separation [27–29]. These nebulizers have been used for routine analyses of simple sample matrices, such as waters, cellular liquids and digested biological and geological samples [30–33]. They were also used previously for the introduction of organic samples, especially for the analysis of petroleum [34–36], organic solvents [37,38] and alcohol fuel [39].

In this work, the use of a microconcentric nebulizer coupled to a cyclonic spray chamber for the introduction of solutions with high contents of formic acid in ICP-MS, without the addition of oxygen, is investigated. After optimization, the method will be applied to the determination of trace elements in certified biological samples, treated with formic acid. To the best of our knowledge, this approach was not used before for the elemental analysis of biological samples. For comparison purpose, the certified samples will be also analyzed by conventional ICP-MS, after digestion.

2. Material and methods

2.1. Instrumentation

An inductively coupled plasma mass spectrometer, *Perkin-Elmer SCIEX*, ELAN 6000 (Thornhill, Canada) was used. The argon gas with minimum purity of 99.996% was supplied by *White Martins* (São Paulo, Brazil). A Microconcentric nebulizer MCN-100 acquired from *CETAC Technologies* (Omaha, USA) was coupled to a labmade cyclonic spray chamber. For the sample flow control, a peristaltic pump from *Ismatec* (Glattbrugg, Switzerland) was used. The sample solution flow rate was 30 μL min^{-1}. The used nebulizer flow rate, 0.7 L min^{-1}, and the RF power, 800 W, were optimized. Tygon orange/red 0.19 mm i.d. and 40 cm length peristaltic pump tubing was used. The instrument conditions were: auto lens mode on, peak hopping measure mode, dwell time of 15 ms, 40 sweeps/reading, 1 reading/replicate, 3 replicates. Platinum sampler and skimmer cones were used. Before changing the to the microconcentric nebulizer, a performance check for sensitivity and oxide and doubly charged ions formation, using a conventional cross flow nebulizer and a Scott spray chamber was carried out. 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 and spray chamber and external calibration with an 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). Concentrated nitric acid (65% v/v) and hydrochloric acid (37% v/v) from *Merck* (Darmstadt, Germany), formic acid (85% v/v) and hydrogen peroxide (40% v/v) from *Vetec* (Rio de Janeiro, Brazil) 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* (Norwalk, USA) , Rh, Y and Ir mono-elemental standard solutions from *Perkin Elmer*, Ba and Ce standards solutions from *SPEX* (Medison, EUA) and Fe and Se mono-elemental solutions from *Merck* (Darmstadt, Germany) were used. The analyzed samples were: Bovine Liver 1577b and Oyster Tissue 1566a from *National Institute of Standards 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

For the optimization study, solutions were prepared with 1% v/v nitric acid and variable concentrations of formic acid (0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90% v/v) and enriched with aqueous standards to a final concentration of 30 μg L^{-1}, except for Se and Fe that were enriched to 300 μg L^{-1}. These solutions were used do investigate the analyte signal intensity in different formic acid concentrations, the signal intensity stability with time and optimization of the nebulizer gas flow rate and of the RF power. The interferences by double charged ions and oxides were verified by using solutions containing 10 μg L^{-1} of Ba and of Ce and increasing concentrations of formic acid (same as above). A blank solution was always measured and taken into consideration. For the investigation of the polyatomic ions, 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 solutions were introduced into the plasma at 30 μL min^{-1} flow rate applying 1100 W RF power and 0.9 L min^{-1} nebulizer gas flow rate before their optimization. The optimized and adopted conditions were RF power of 800 W and nebulizer gas flow rate of 0.7 L min^{-1}.

Using an analytical balance AG204 from *Mettler Toledo* (Giessen, Germany), approximately 75 mg of each sample were exactly 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 was kept at 90 °C in a water bath, during one hour. Then, the volume was completed to 10 mL with water and nitric acid to a 1% v/v final concentration was added. After the treatment, the solutions were dark color with a high intensity formic acid smell, however, after the dilution, the smell was much weaker. 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.

For the external calibration against aqueous standards, the standard solutions were prepared in 1% v/v nitric acid. For the calibration in the organic medium, the solutions were prepared in 50% v/v formic acid and 1% v/v nitric acid. The analytes concentrations were 0.5; 1; 2.5; 5; 10, 25 and 50 μg L^{-1}, except for Fe which the used concentrations were 10, 25, 50, 100, 250, 500, and 1000 μg L^{-1}. As internal standard, Rh was added to all solutions, including the samples solutions, to a 5 μg L^{-1} final concentration.

3. Results and discussion

3.1. Effect of formic acid on analyte sensitivity

When introducing solutions with high organic content, it is important to verify the effect of the organic load on the signal intensity, in order to use an appropriate calibration strategy for accurate analysis. Several authors [17,18,39] have observed that small amounts of organic compounds in the plasma can increase the

nebulization and aerosol transport efficiencies, increasing the signal in relation to that for aqueous solution. Organic compounds can also improve the ionization efficiency of high ionization energy elements by carbon charge transfer reactions [17,18]. However, by increasing the content of carbon in the solution, the inverse effect can happen due to the instability of the plasma and deposition of carbon on the cones and lens [18,19,39].

The signal intensities of the different analytes in relation to the formic acid content, for the same analyte concentration, are shown in the Fig. 1a. The signal intensities increase for formic acid concentrations up to 90%v/v. However, for all formic acid contents, the signal intensities in this medium are always higher than those in water without organics, justifying its use, when sensitivity is critical. Probably, a more efficient nebulization and transport of the aerosol of formic acid solutions can explain the sensitivity increase, as the formic acid has a lower surface tension than water, favoring the formation of a finer aerosol that reach the plasma. The surface tension of the water is 71.13 mN m^{-1}, while that of the concentrated formic acid is 37.13 mN m^{-1}, both at 25 °C [40]. The maximum increase in the analytes signal, in relation to those in aqueous solution without any organic constituent, is from 2.4 to 3.3 times. However, for As, Se and Zn, the increase was approximately 4.5, 7.8 and 9 times more than in water, respectively. Higher sensibility increases were observed for analytes with ionization potential above 9 eV, as shown in Fig. 1b. As already mentioned, these analytes are only partially ionized in the plasma conditions and their ionization is improved in the presence of carbon due to transfer charge reactions between charged carbon species and the analytes [17,18]. For elements of lower ionization potential, the increase, around 2.5 times, is about the same for all elements independently of the their ionization potential and can be explained by the more efficient sample transport to the plasma, as discussed above.

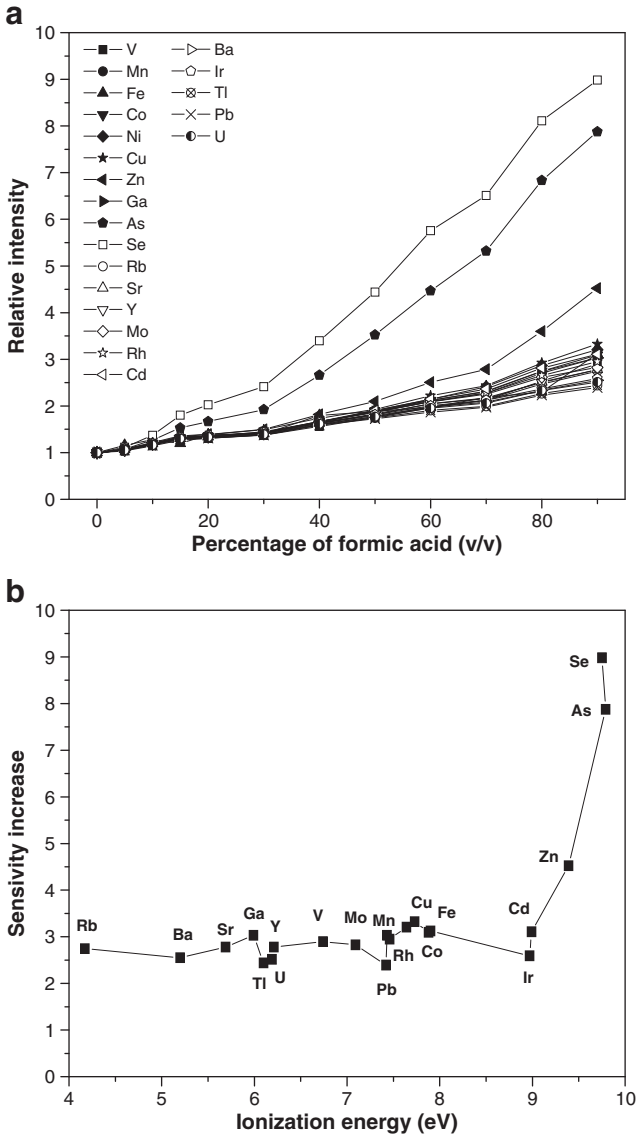

Fig. 1. (a) Effect of formic acid content on the relative signal intensity of the analytes; (b) sensibility increase versus the analyte ionization potential (eV). Analyte concentrations: 300 μg L^{-1} (Se and Fe) or 30 μg L^{-1} (all others). The signal intensity was related to the value for the solution without formic acid. (RF power: 1100 W, nebulizer gas flow rate: 0.9 L min^{-1}, sample flow rate: 30 μL min^{-1}).

3.2. Effect of formic acid on interference

The instrument manufacturer recommends a limit of 3% for the CeO^+/Ce^+ and Ba^{++}/Ba^+ ratios in order to reduce spectral interference caused by oxides and double charged ions. As shown in Fig. 2, in the absence of formic acid and for up to 80% formic acid, both ratios are lower than 3%. For acid concentrations from 40%v/v to 90%v/v, the ratio Ba^{++}/Ba^+ increases pronouncedly, probably due to charge transfer reactions between carbon charged species and the ion Ba^+, but remains below the limit for formic acid concentrations up to 80%v/v. On the other hand, formic acid has no significant effect on the $CeO^+/Ce+$ ratio that remains between 0.75% and 1% for all studied formic acid concentrations. It should be pointed out that the position of the capillary in relation to the sapphire orifice of the microconcentric nebulizer is very critical. A small movement of the capillary may change the instrument performance dramatically in relation to the analyte, oxides and double charged ions intensity signals.

This study was carried out using a RF power of 1100 W and a nebulizer gas flow rate of 0.9 L min^{-1}. However, for a solution with 50%v/v formic acid, it was found that the CeO^+/Ce^+ and Ba^{++}/Ba^+ ratios are constant and below 0.5% for nebulizer gas flow rates between 0.6 and 0.9 L min^{-1}. For lower gas flow rates, the ratios increase as the flow rate decreases. By increasing the power, from 800 W to 1300 W, there was a very small increase in the ratios, remaining below 1%, which is less than the acceptable limit of 3% for our ICP-MS instrument.

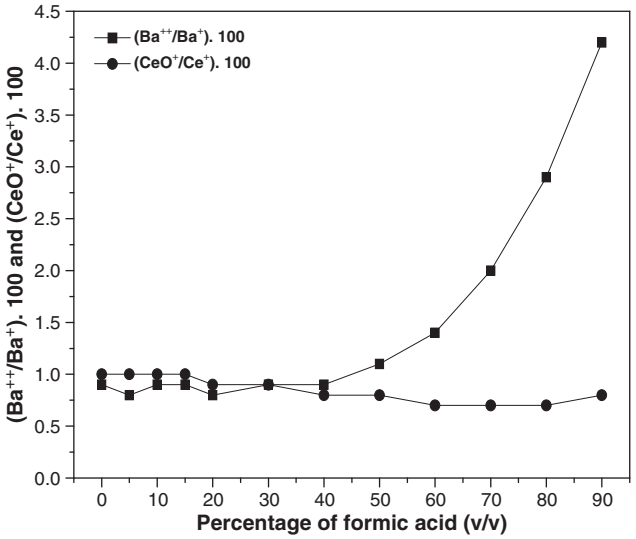

Fig. 2. Effect of formic acid on the $^{140}Ce^{16}O^+/^{140}Ce^+$ ratio and on the $^{138}Ba^{++}/^{138}Ba^+$ ratio. The solution contained 10 μg L^{-1} of Ba and of Ce. The signal intensity was related to the value for the solution without formic acid. (RF power: 1100 W, nebulizer gas flow rate: 0.9 L min^{-1}, sample flow rate: 30 μL min^{-1}.

Some typical argon, oxygen, chlorine and nitrogen polyatomic ions were investigated by introducing solutions containing different formic acid concentrations and 0.1%v/v HCl. As shown in the Fig. 3a and b, the signal intensities of $^{14}N^{35}Cl^+$, $^{14}N^{12}C^+$, $^{40}Ar^{12}C^+$, $^{13}C^{37}Cl^+$, $^{40}Ar^{35}Cl^+$ and $^{36}Cl^{16}O^+$ increase in comparison to that for an aqueous solution without formic acid, but with 1.0%v/v hydrochloride acid and 1.0% nitric acid (signal intensities of 128, 229, 8634, 66, 745, 9468, 32421 and 314 cps, respectively). It should be noted that up to 50%v/v formic acid, the increase is small. For formic acid contents above 50% v/v, the increase becomes important, reaching, 10 times for $^{36}Cl^{16}O^+$ and $^{40}Ar^{35}Cl^+$, 450 times for $^{40}Ar^{12}C^+$, 1200 times for $^{14}N^{35}Cl^+$ and $^{13}C^{37}Cl^+$ and 6000 times for $^{14}N^{12}C^+$ at a formic acid concentration of 90%v/v. Possibly, charge transfer reactions from carbon increase the nitrogen and chlorine polyatomic ions. The formic acid effect on $^{40}Ar^{18}O^+$ ions was much less intense and on $^{40}Ar^{36}Ar^+$ was negligible. Anyway, for a formic acid concentration of 50%v/v, used in this work, the most critical polyatomic ions have a low population, similar as for aqueous solution without formic acid. In conclusion, it seems that spectral interference is negligible in the presence of 50%v/v formic acid. It was found, in a separated study, that the Ca polyatomic ion (CaOH) interference on ^{57}Fe was negligible when the microconcentric nebulizer was used, independently of the Ca concentration, but increased for formic acid concentrations higher than 50%v/v. Details of this study will not be shown in the manuscript.

In the Fig. 4, the effects of: (a) the nebulizer gas flow rate and (b) RF Power on the polyatomic ions are presented. Fig. 4a shows the increasing intensities of chlorine and carbon polyatomic ions with the nebulizer gas flow rate especially up to 0.6 L min^{-1}, except for $^{40}Ar^{36}Ar^+$ and $^{40}Ar^{18}O^+$. For these two ions, the signal intensities decrease up to 30% for a flow rate of 0.6 L min^{-1}. This decrease could be due to competition for the carbon by Ar and O to form the respective polyatomic species and/or to the cooling of the plasma. For higher flow rates, up to 0.9 L min^{-1}, the $^{40}Ar^{36}Ar^+$ and $^{40}Ar^{18}O^+$ populations remain almost constant. As shown in Fig. 4b, the signal intensities of all studied polyatomic ions increase with the RF power, meaning that the polyatomic ions are more effectively formed in the more energetic plasma. Concerning elimination of interference by polyatomic ions, the best conditions were low RF power and nebulizer gas flow rate above 0.6 L min^{-1}. Analysis of certified reference

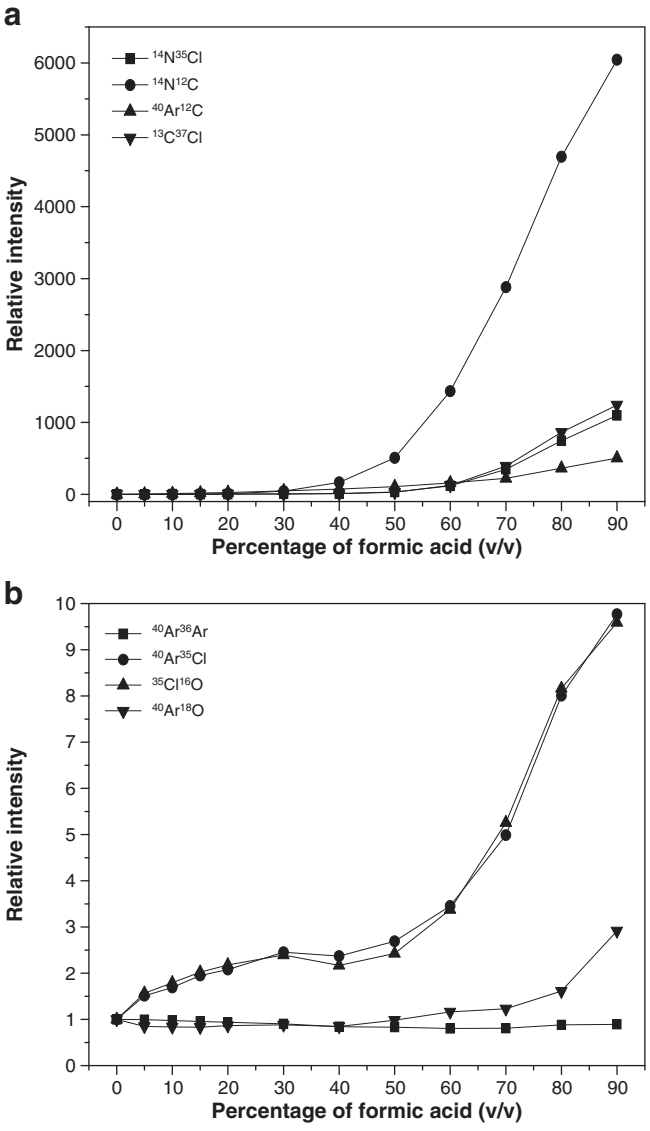

Fig. 3. Effect of formic acid on: (a) $^{14}N^{12}C^+$, $^{40}Ar^{12}C^+$, $^{13}C^{37}Cl^+$ and $^{14}N^{35}Cl^+$ polyatomic ions; (b) $^{40}Ar^{36}Ar^+$, $^{40}Ar^{18}O^+$, $^{40}Ar^{35}Cl^+$ and $^{36}Cl^{16}O^+$ polyatomic ions. The signal intensity was related to the value for the solution without formic acid. (RF power: 1100 W, nebulizer gas flow rate: 0.9 L min^{-1}, sample flow rate: 30 μL min^{-1}).

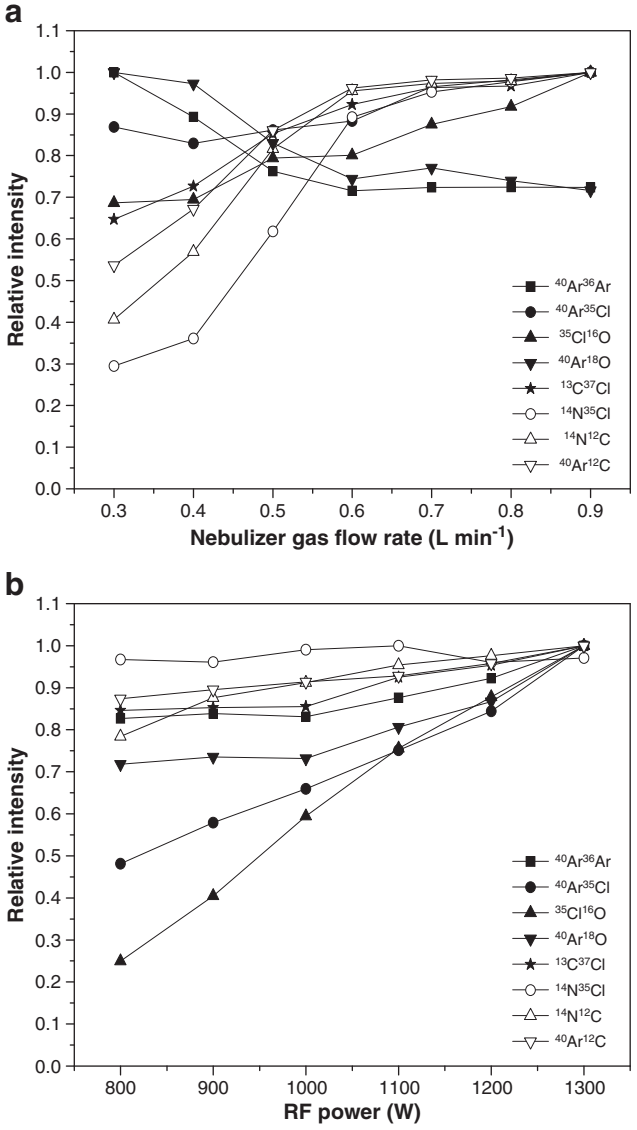

Fig. 4. Effect of nebulizer gas flow rate (a) and RF Power (b) on polyatomic ions formation for an aqueous solution containing 50%v/v of formic acid, 0.1% hydrochloric acid and 1%v/v nitric acid. The signal intensity was related to the higher value obtained for each curve. (Nebulizer gas flow rate of 0.9 L min^{-1} for the RF power optimization and RF Power of 1100 W for the nebulizer gas flow rate. Sample flow rate: 30 μL min^{-1}).

material by the proposed procedure and also after sample digestion will also demonstrate the absence of interference.

3.3. Nebulizer gas flow rate and RF power optimization

Fig. 5 shows the analyte relative sensitivity as the nebulizer gas flow rate increases or as the RF power increases for a standard aqueous solution containing the analytes, without (Fig. 5a and b) and with 50% v/v of formic acid (Fig. 5c and d). The signal intensities increase for both solutions as the flow rate increases up to 0.7 L min^{-1} (without formic acid) or up to 0.6 L min^{-1} (with formic acid) and then remain almost constant for higher flow rates. A nebulizer flow rate of 0.7 L min^{-1} was adopted for further experiments. The different physical properties of the solutions may explain the lower flow rate required reaching the optimum sensitivity for the solution with formic acid due to a more efficient nebulization and, consequently, a more efficient analyte transport to the plasma, as already explained. Concerning the RF power, it is clear that, at 800 W RF power, better sensitivity was obtained for the majority of the analytes in both standard solutions (without and with formic acid). This power that characterizes a cold plasma was adopted in this work. The decrease of the analyte signals for RF power above 800 W, is generally more intense for the solution containing formic acid, as higher energy consumption is required to break the organic molecules in comparison to the water molecules. The increase of double ionization with the RF can explain the decrease in sensitivity for higher RF power. It is interesting to note that for high ionization energy elements, such as As, Cd, Se and Zn, there was a slight increase in the sensitivity with the RF power only for the solution without formic acid (Fig. 5b). In the presence of formic acid, the sensitivities for these elements are much higher, as it was already discussed. As also shown in the Fig. 5, the ^{56}Fe signal intensity has a singular behavior, having a higher signal intensity for lower nebulizer flow rates (Fig. 5a and c) and for higher RF power (Fig 5d) in comparison to the other analytes. Higher signal intensity could be produced by spectral interference. Anyway, it has been shown that cold plasma conditions are the best for the ^{56}Fe determination by ICP-MS [41].

The conditions established in this work, especially the RF power, are very different from those indicated in other works [17–19] that employed conventional nebulizers to introduce organic solutions. The

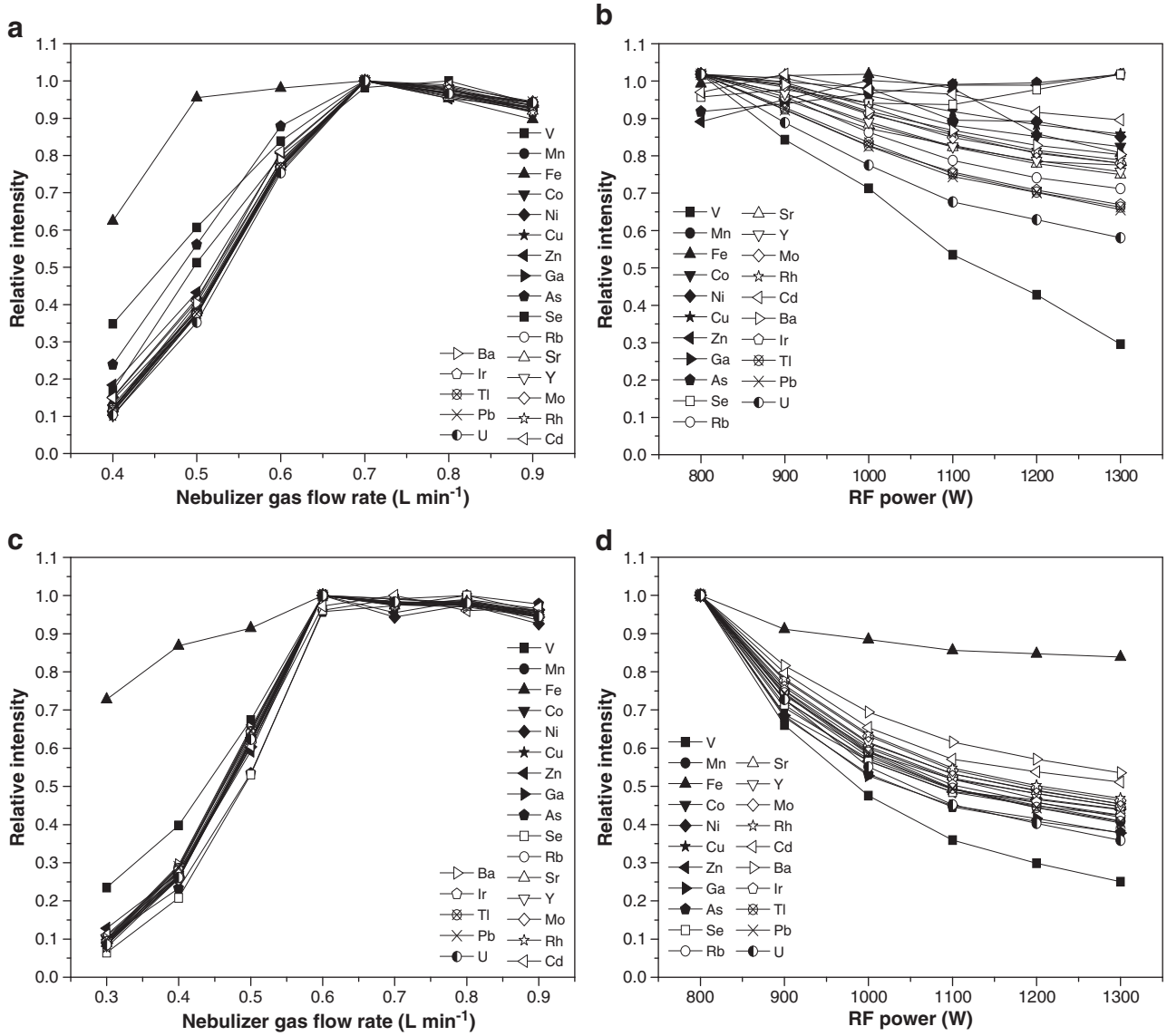

Fig. 5. Effect of the nebulizer gas flow rate and of the RF power on the relative analyte signal intensity for an aqueous standard solution without formic acid (a and b) and with 50% v/v formic acid (c and d). Analyte concentration: 300 μg L^{-1} (Se and Fe) or 30 μg L^{-1} (all others). The signal intensity was related to the higher value obtained for each curve. (Sample flow rate: 30 μL min^{-1}). RF power of 1100 W for Fig. 5a and c and nebulizer gas flow rate of 0.9 L min^{-1} for Fig. 5b and d.

use of a micronebulizer allows the introduction of very small amount of the sample solution into the plasma, avoiding undesirable effects, using a low RF Power. As the sample amount is low, the plasma has enough energy to decompose the introduced formic acid and the sample matrix, keeping the plasma stability. Besides, formic acid has considerable higher oxygen content in relation to carbon content in its composition. During its decomposition in the plasma, the oxygen may react with the carbon, avoiding carbon deposition on interface parts. No carbon deposit on cones was verified after running the instrument for 8 h.

3.4. Analytical application

The formic acid treatment of biological samples should be carried out under heating, in order to accelerate the sample dissolution, otherwise the procedure is very slow. Previous procedure [8,9] uses heating at 50 °C for 2 to 4 h in an ultrasonic bath for the dissolution of biological materials. However, we observed that the solubilization time can be decreased to one hour if the temperature is increased to 90 °C, without ultrasonic treatment, even when a microconcentric nebulizer is used. Another difference is that our procedure requires the addition of 1% v/v nitric acid. Solid particles in the sample solution may clog the microconcentric nebulizer if the solubilization was carried out at temperatures lower than 80 °C and the sample solution is too viscous. Tissues with cellulose and lignin are not completely solubilized, for this reason, botanical samples could not be analyzed using the proposed procedure due to micronebulizer blockage.

In Table 1, the method figures of merit are shown. External calibration with standard solutions in 50% formic acid, using $^{103}Rh^{+}$ as internal standard, was carried out for the proposed method. For the analysis of the digested sample, the same calibration, but without formic acid in the standard solutions, was used. The detection limit (LOD) was defined as 3 times the standard deviation of ten measurements of the blank concentration (50% v/v of formic acid for the proposed procedure or the digestion blank for conventional procedure), multiplied by the sample dilution factor (133 times for the proposed method and 1000 times for the digestion procedure). The obtained limits show that the proposed method has appropriate detection capability for trace element analysis, being the highest value of 220 μg kg^{-1} for Fe and the smallest of 0.5 μg kg^{-1} for Tl. The high value for Fe can be attributed to the low abundance of the measured isotope (2.2%) and to the relatively high background. The LOD values for the conventional introduction system were very similar to the ones of the proposed method. The LOD values obtained for the digested sample analysis using a microconcentric nebulizer, also shown in Table 1, were relatively higher due the high sample dilution factor when compared to those obtained with the proposed method. They are also higher, when compared to those obtained by the conventional introduction system due to the lower sample flow rate, among other possible reasons. The limit of quantification defined as 3.3 times the LOD, can be easily obtained from the LOD values in Table 1.

Table 1
Figures of merit for the trace elements determination in biological samples treated with formic acid using a microconcentric nebulizer as introduction sample system (proposed method) or after sample digestion using a conventional nebulizer (conventional method). N = 3.

Analyte	Isotope (u.m.a)	Proposed method (formic acid treatment)		Conventional method (sample digestion)		Microconcentric nebulizer (sample digestion)	
		LOD (μg kg^{-1})*	RSD (%)	LOD (μg kg^{-1})*	RSD (%)	LOD (μg kg^{-1})	RSD (%)
V	51	3.0	0.5	4.5	1.0	1000	8.1
Mn	55	4.0	0.2	20	0.3	100	1.0
Fe	57	220	1.5	710	0.7	4000	3.1
Co	59	1.0	1.4	3.0	2.1	30	8.3
Ni	60	10	1.0	8.0	1.3	200	8.3
Cu	63	5.0	0.5	10	1.0	80	0.7
Zn	66	30	0.2	25	1.3	300	1.0
Ga	69	1.5	3.5	1.5	2.7	50	150
As	75	1.5	0.3	9.0	1.7	750	3.2
Se	82	20	0.7	100	4.2	3500	4.6
Rb	85	1.0	0.8	1.5	1.0	20	2.1
Sr	88	2.5	0.2	2.0	1.1	15	0.9
Mo	98	10	1.1	5.0	1.1	200	11.0
Cd	111	3.0	0.5	3.5	1.9	100	3.3
Ba	138	1.5	1.0	2.5	1.0	20	1.6
Tl	205	0.5	2.9	0.5	1.7	7.5	43.5
Pb	208	1.5	0.7	10	1.1	30	2.8
U	238	1.0	0.5	0.5	1.1	10	4.1

* 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.

The relative standard deviation (RSD, n = 3) of the concentrations in Oyster Tissue solution, used to estimate the precision, was smaller than 3.5% for all of the analytes, including elements commonly subjected to polyatomic ions interferences, such as Fe, Ni and Se. The precision of the proposed method was usually equal or better than that obtained for7 the analysis of the digested samples using a conventional introduction sample system. The obtained precision is really surprising, and probably not attainable using conventional nebulizers for solutions with high dissolved solid and high carbon contents. It has to be mentioned that ^{103}Rh, used as internal standard, certainly contributes to the excellent precision. The figures of merit obtained using calibration with aqueous standard without formic acid were very similar and therefore are not shown in this paper.

For the analytes quantification, external calibrations without formic acid or external calibration with 50% v/v formic acid, both using ^{103}Rh as internal standard, were tested for four certified reference materials, as shown in Table 2. The reference materials are not certified for all determined elements. For the non certified elements, the analyte concentrations was obtained by the analysis of the digested samples, using a conventional introduction system; they are expressed by mean ± confidence interval of 95% using the t-test and they are differentiated in the table by an asterisk. Accuracy was the main criterion for selecting the best conditions for sample analysis. By comparing the obtained results with the certified values, using the t-test at a 95% confidence level, the aqueous calibration can be used for the determination of several analytes, mainly those that have low ionization energy. This shows that potential non-spectral interference, such as those related to the nebulization and to the solution transport are compensated by the use of the internal standard. However this is not true for As, Se and Zn as can be observed in the supplementary electronic material, where the logarithm of the obtained values were plotted in function of the logarithm of the certified values (the dotted line is for a correlation coefficient of one). Due to the higher ionization energy of these elements compared to that of Rh, the charge transfer ionization is more effective for As, Se and Zn than for Rh in the solutions containing formic acid. However, if the calibration in the presence of formic acid is employed, the effect of the charge transfer ionization happens at similar intensities in the standards and in the samples, explaining the agreement of the obtained concentrations with the certified values for most analytes. In addition, the linear correlation coefficients (r) are much closer to one for the calibration with standard solutions containing formic acid using Rh as internal standard, indicating a better accuracy for this calibration strategy, which is recommended. Another advantage of the procedure is that it does not require the use of liposoluble stock standards solutions.

4. Conclusions

Using a microconcentric nebulizer operated at low flow rate, it is possible to introduce sample solutions with high formic acid and dissolved solids contents. Generally, in formic acid solution the

Table 2
Obtained concentrations (mg kg^{-1}) in the certified reference materials treated with formic acid using calibration against aqueous standard solutions without formic acid and with 50% v/v formic acid. N = 3.

	TORT 2			Bovine liver 1577b			Oyster tissue 1566a			Dolt 3		
	Certified/digested*	Without formic acid	With formic acid	Certified/digested*	Without formic acid	With formic acid	Certified/digested*	Without formic acid	With formic acid	Certified/digested*	Without formic acid	With formic acid
V	1.64 ± 0.19	1.77 ± 0.06	1.56 ± 0.03	0.123	0.122 ± 0.043	0.099 ± 0.019	4.68 ± 0.15	4.66 ± 0.12	4.22 ± 0.03	0.67 ± 0.03*	0.444 ± 0.130	0.691 ± 0.375
Mn	13.6 ± 1.2	13.8 ± 2.4	11.9 ± 0.9	10.5 ± 1.7	11.1 ± 0.4	9.89 ± 0.92	12.3 ± 1.5	13.7 ± 0.3	10.6 ± 0.1	10.1 ± 2.1*	10.4 ± 0.3	9.37 ± 0.61
Fe	105 ± 13	64.5 ± 5.4	93.3 ± 21.6	184 ± 15	127.9 ± 31.8	157.5 ± 28.9	539 ± 15	265.9 ± 3.9	329.1 ± 12.0	1484 ± 57	1275.2 ± 43.3	1490.5 ± 34.4
Co	0.50 ± 0.09	0.500 ± 0.060	0.451 ± 0.040	250	0.230 ± 0.003	0.224 ± 0.021	0.570 ± 0.110	0.282 ± 0.047	0.363 ± 0.073	0.293 ± 0.035*	0.317 ± 0.012	0.258 ± 0.015
Ni	2.50 ± 0.20	2.61 ± 0.40	2.15 ± 0.19	0.271 ± 32*	0.268 ± 0.032	0.288 ± 0.067	2.25 ± 0.44	2.76 ± 0.83	2.19 ± 0.58	2.72 ± 0.35	2.33 ± 0.12	2.32 ± 0.27
Cu	106 ± 10	108 ± 17	91.2 ± 7.5	160 ± 8	172 ± 6.2	149 ± 13	66.3 ± 4.3	77.9 ± 0.4	58.0 ± 1.7	31.2 ± 1.0	34.4 ± 1.3	30.3 ± 1.2
Zn	180 ± 6	253 ± 44	163 ± 2	127 ± 16	174 ± 6	114 ± 4	830 ± 57	1536 ± 143	7890 ± 43	86.6 ± 2.4	12.9 ± 3.2	85.3 ± 3.6
Ga	0.030 ± 0.006*	0.036 ± 0.006	0.034 ± 0.004	< LOD*	< LOD	< LOD	0.060 ± 0.014*	0.055 ± 0.008	0.049 ± 0.005	0.007 ± 0.001*	0.016 ± 0.007	0.008 ± 0.005
As	21.6 ± 1.8	58.6 ± 10.1	19.1 ± 0.3	0.050	0.251 ± 0.093	0.054 ± 0.006	14.0 ± 1.2	49.2 ± 0.5	12.6 ± 0.4	10.2 ± 0.5	26.7 ± 0.9	8.60 ± 0.16
Se	5.63 ± 0.67	21.5 ± 2.4	4.79 ± 0.14	0.730 ± 0.060	3.14 ± 0.72	0.742 ± 0.111	2.21 ± 0.24	9.71 ± 0.32	2.03 ± 0.13	7.06 ± 0.48	27.5 ± 2.03	6.38 ± 0.10
Rb	2.48 ± 0.05*	2.28 ± 0.38	2.31 ± 0.19	13.7 ± 1.1	11.6 ± 0.3	11.9 ± 1.1	2.80 ± 0.03*	2.63 ± 0.01	2.70 ± 0.04	3.09 ± 0.07*	2.99 ± 0.04	3.07 ± 0.10
Sr	45.2 ± 1.9	36.7 ± 6.0	37.2 ± 0.8	0.136 ± 0.001	0.126 ± 0.008	0.129 ± 0.009	11.1 ± 1.0	9.31 ± 0.09	9.61 ± 0.04	3.75 ± 0.08*	3.51 ± 0.10	3.57 ± 0.02
Mo	0.95 ± 0.11	0.942 ± 0.059	0.826 ± 0.062	3.5 ± 0.3	3.23 ± 0.02	3.26 ± 0.31	0.162 ± 0.014*	0.146 ± 0.030	0.190 ± 0.028	3.04 ± 0.15*	2.80 ± 0.11	2.79 ± 0.61
Cd	26.7 ± 0.6	27.8 ± 4.9	24.35 ± 1.64	0.5 ± 0.1	0.521 ± 0.037	0.477 ± 0.120	4.15 ± 0.38	4.61 ± 0.17	3.99 ± 0.14	19.4 ± 0.6	20.3 ± 0.4	18.08 ± 0.44
Ba	1.68 ± 0.04*	1.34 ± 0.18	1.46 ± 0.13	0.044 ± 0.007*	0.043 ± 0.009	0.047 ± 0.008	1.7 ± 0.3*	0.885 ± 0.304	1.58 ± 0.19	0.153 ± 0.039*	0.122 ± 0.043	0.127 ± 0.031
Tl	0.012 ± 0.001*	0.0130 ± 0.0053	0.0140 ± 0.0031	0.0034 ± 0.0003*	0.0042 ± 0.0019	0.0050 ± 0.0008	0.0064 ± 0.0013*	0.0047 ± 0.0012	0.0057 ± 0.0006	0.0119 ± 0.0006*	0.0112 ± 0.0038	0.0110 ± 0.0032
Pb	0.350 ± 0.130	0.298 ± 0.013	0.313 ± 0.054	0.129 ± 0.004	0.104 ± 0.049	0.118 ± 0.015	0.371 ± 0.014	0.253 ± 0.012	0.357 ± 0.015	0.320 ± 0.050	0.267 ± 0.047	0.315 ± 0.064
U	0.029 ± 0.001*	0.035 ± 0.002	0.031 ± 0.005	<LOD*	<LOD	<LOD	0.132 ± 0.012	0.080 ± 0.001	0.116 ± 0.013	0.022 ± 0.001	0.025 ± 0.002	0.020 ± 0.006

* Non-certified values obtained after microwave assisted sample digestion and determination using a conventional introduction sample system.

sensitivity is higher than in aqueous solution without formic acid due to the more efficient sample nebulization and aerosol transport. The optimum nebulizer gas flow rate and the RF power are much smaller than those normally used with conventional nebulizers. The sample treatment with formic acid is fast and easy at 90 ºC. The quantification by external calibration using standards solutions without formic acid and with $^{103}Rh^{+}$ as internal standard can be used for analytes with low ionization energy, otherwise, the calibration with 50% v/v formic acid solution, is required. In general, external calibration with aqueous standard solutions containing 50% v/v formic acid resulted in better accuracy, lower detection limits, and very low relative standard deviations for most of the analytes, what is surprising for a low flow rate micronebulizer. The proposed method is precise, accurate, less expensive when compared to acid digestion, for example, and allows a high sample throughput. In addition, undesirable effects of the organic load in the plasma, such as carbon deposits and plasma instability are avoided.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.sab.2010.09.007.

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