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Micronebulization for trace analysis of lanthanides in small biological specimens by ICP-MS

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Abstract

This work deals with the development of a mass spectrometric method for the determination of lanthanides (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu) at ng g^{-1} levels in small amounts of biological specimens by inductively coupled plasma-mass spectrometry (ICP-MS) after microwave digestion. Two different systems were investigated for introducing the aqueous solutions of the sample into the plasma: a desolvating system (APEX) with micronebulizer and a nano-volume flow injection system combined with a total consumption nebulizer (DS-5). Both solution introduction systems were used together with a quadrupole ICP mass spectrometer. The performances of the investigated nebulizers were compared to that of the MicroMist nebulizer, which was fitted to a mini cyclonic spray chamber. The solution uptake rate was: 700 μL min^{-1} for MicroMist, 330 μL min^{-1} for APEX and 8 μL min^{-1} for DS-5. By using the APEX and the DS-5 nebulizers the oxides formation rate is reduced compared to MicroMist nebulizer, but to a larger extent by APEX. The relative detection limits for lanthanides ranged from 0.57 to 6.1 ng L^{-1} and 30 to 170 ng L^{-1} for the APEX and the DS-5 nebulizer, respectively. The absolute detection limits were in the range of 6.7 to 54 pg for APEX and 3.1 to 7.6 fg for DS-5. The method was applied for lanthanides determination in mussel tissue (BCR 668) and in slugs organs. Good precision and accuracy were obtained with the use of APEX, since the oxide interference is markedly reduced. Slight interference was still observed with the use the DS-5 nebulizer, mainly by Ba oxides. By using the nano-volume flow injection nebulizer, lanthanide determination in small amounts of slug tissue was possible, only requiring 76 nL of digested sample solution into the plasma of ICP-MS.

Keywords: ICP-MS; Flow injection; Micronebulizers; Lanthanides; Small biological tissues: Trace analysis

1. Introduction

Lanthanides, because of their diversified physical and chemical properties, have been widely used in a number of fields (e.g., in microelectronics and optics, in material science or in nuclear reactors, in biomolecular research or in medical diagnostics) [1–3]. As a result of their increasing application, more and more lanthanides are entering into the environment, and animal and human bodies. It has already been observed that Ce, Pr and Lu can accumulate in the liver affecting cell physiology. These effects appeared to result from the similarity of lanthanides cationic radii to the size of Ca^{2+} ions. Trivalent lanthanide ions, especially La^{3+} and Gd^{3+}, block different calcium channels in human and animal cells and also affect numerous enzymes [4,5]. Furthermore, the biological effects of the lanthanides in living organisms, based on their similarity to calcium, have stimulated researches into their therapeutic application [6]. Recently, a new compound, lanthanum carbonate, has been approved as phosphate binding agent in chronic renal failure [6]. A group of lanthanide complexes, the texaphyrins, has recently progressed into clinical trials for the treatment of atherosclerosis, rheumatoid arthritis and cancer [6]. Consequently, the determination of lanthanides at trace concentration levels in biological systems is attaining increasing importance. However, the elements are chemically and physically similar, have complex emission and absorption spectra and are generally present at the sub ng g^{-1}

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level in biological samples. Therefore, multi-element determination of all lanthanides usually presented difficulties before the advent of inductively coupled plasma mass spectrometry (ICP-MS) [7]. Nowadays, due to its high sensitivity, ICP-MS seems to be the more appropriate technique for lanthanides determination at trace levels. However, accurate determination is still not easy owing to interference from isobaric atomic ions and polyatomic ions, mainly oxides like those shown in Table 1. The difficulty involved in this determination will be increased when the concentration ratio of lighter lanthanide to heavier lanthanide is high. Furthermore, Ba is usually found in a relative high concentration in environmental, geological and biological samples and, therefore, its oxides and hydroxides can interfere, especially on Nd, Sm, Eu and Gd [8]. With a maximum mass resolution of 10.000 for double-focusing ICP mass spectrometers, the isobaric interferences of atomic and oxide ions can be separated (Table 1). In practice, the theoretically required mass resolution is insufficient if the intensity of oxide ions is significantly higher than that of the analyte ions [7,9]. Augagneur et al. [10] determined lanthanides in wine and observed that the double focused sector field ICP-MS instrument they used was not powerful enough to resolve the isobaric interferences of lanthanide oxide ions on analyte ions. Other alternatives should be used in order to resolve the interference problems, as for instance matrix separation [11,12] and/or using water desolvation-nebulizers systems [13]. In this type of nebulizer, the water solvent molecules that enter the plasma can be dramatically reduced and, therefore, oxide and hydroxide formation can be minimized. In addition, the detection limit is improved [14]. By using a membrane desolvation-nebulizer (APEX), D'Ilio et al. [13] observed that the formation of the following interfering oxide and hydroxide species were remarkably reduced: $^{11}B^{16}O^+$ on $^{27}Al^+$, $^{37}Cl^{16}O^+$ on $^{53}Cr^+$, $^{37}Cl^{16}OH^+$ on $^{54}Cr^+$, $^{37}Cl^{18}O^+$ and $^{39}K^{16}O^+$ on $^{55}Mn^+$, $^{43}Ca^{16}O^+$ on $^{59}Co^+$, $^{43}Ca^{16}OH^+$ on $^{60}Ni^+$, for samples with high Ca content, and $^{66}Zn^{16}O^+$ on $^{82}Se^+$. The hydrides formation rate may be reduced as well. By employing an APEX nebulizer for the determination of uranium in aqueous solution, Boulyga and Heumann [15] observed that the ratio UH^+/U^+ were less than 10^{-6}.

Trace elements determination in small amounts of environmental and biological samples may require special sample preparation and measurement techniques [18], mainly when the amount of material is limited. In this case, efficient sample introduction systems are advantageous, especially if the analyte concentration is very low and a dilution of the sample solution results in deterioration of detection limit. The high-efficiency micronebulizers are useful for the determination of essential and toxic trace elements in biological materials such as body fluids, human or animal tissues, organs, etc. where ICP-MS is used mostly as a sensitive multielemental trace and ultra trace analytical technique. With the use of micronebulizers for introducing the sample solution into the ICP-MS plasma, it is possible to determine trace elements in few mg of digested solid sample. Different nebulizers for the introduction of radioactive sample solution into the plasma were investigated by Becker and Dietze [19]. By using the direct injection high-efficiency nebulizer (DIHEN) with a solution uptake rate of 85 μL min^{-1}, the sensitivity achieved for radionuclides (^{232}Th, ^{237}Np, ^{238}U,

Table 1
Lanthanides measured, isotope abundances and possible interferences in ICP-MS [16,17]

Element	*m/z*	Abundance (%)	Interfering species	Element	*m/z*	Abundance (%)	Interfering species
La	139	99.91		Gd	158	24.84	$^{142}Ce^{16}O^+$ (8000), $^{142}Nd^{16}O^+$ (7385)
Ce	140	84.48		Gd	160	21.86	$^{150}Sm^{16}O^+$ (7942), $^{144}Nd^{16}O^+$,$^{160}Dy^+$ (86168)
Ce	142	11.08	$^{142}Nd^+$ (93238)	Tb	159	100	$^{143}Nd^{16}O^+$ (10226)
Pr	141	100		Dy	161	18.9	$^{145}Nd^{16}O^+$ (8276)
Nd	142	27.13	$^{142}Ce^+$ (93238)	Dy	162	25.5	$^{146}Nd^{16}O^+$ (8628),$^{162}Er^+$ (81700)
Nd	144	23.80	$^{144}Sm^+$ (71149)	Dy	163	24.9	$^{147}Sm^{16}O^+$ (8618)
Nd	146	17.19	$^{130}Ba^{16}O^+$ (12241)	Dy	164	28.2	$^{148}Sm^{16}O^+$ (8434), $^{164}Er^+$ (5854615)
Nd	148	5.76	$^{132}Ba^{16}O^+$ (8927)	Ho	165	100	$^{149}Sm^{16}O^+$ (9050)
Nd	150	5.74	$^{134}Ba^{16}O^+$(6978)	Er	166	33.6	$^{150}Nd^{16}O^+$ (11453)
Sm	147	15.0	$^{130}Ba^{17}O^+$ (15445)	Er	167	22.95	$^{151}Eu^{16}O^+$ (9605)
Sm	148	11.3	$^{132}Ba^{16}O^+$(9954), $^{148}Nd^+$ (71456)	Er	170	14.9	$^{154}Gd^{16}O^+$ (8944), $^{154}Sm^{16}O^+$ (9211), $^{170}Yb^+$ (236021)
Sm	149	13.8	$^{132}Ba^{17}O^+$ (10824)	Tm	169	100	$^{153}Eu^{16}O^+$ (9354)
Sm	150	7.4	$^{134}Ba^{16}O^+$(8388), $^{150}Nd^+$(41482)	Yb	170	3.05	$^{154}Gd^{16}O^+$ (8963), $^{154}Sm^{16}O^+$ (9316), $^{170}Er^+$ (236021)
Sm	152	26.7	$^{136}Ba^{16}O^+$ (7498), $^{136}Ce^{16}O^+$ (8594)	Yb	171	14.3	$^{155}Gd^{16}O^+$ (9899)
Sm	154	22.7	$^{138}Ba^{16}O^+$(7027), $^{138}Ce^{16}O^+$ (7225)	Yb	172	21.9	$^{156}Gd^{16}O^+$ (9155)
Eu	151	47.8	$^{135}Ba^{16}O^+$ (7892)	Yb	173	16.12	$^{157}Gd^{16}O^+$ (8983)
Eu	153	52.2	$^{137}Ba^{16}O^+$ (7416)	Yb	174	31.8	$^{158}Gd^{16}O^+$ (8767)
Gd	155	14.80	$^{139}La^{16}O^+$ (7254)	Lu	175	97.41	$^{159}Tb^{16}O^+$ (8555)
Gd	156	20.47	$^{140}Ce^{16}O^+$ (7162)	Lu	176	2.59	$^{160}Gd^{16}O^+$ (8504), $^{160}Dy^{16}O^+$(7823), $^{176}Yb^+$(1491040), $^{176}Hf^+$ (138537)
Gd	157	15.65	$^{141}Pr^{16}O^+$ (7335)	Yb	176	12.7	$^{160}Gd^{16}O^+$ (8541), $^{160}Dy^{16}O$ (7865), $^{176}Lu^+$ (1630435)

Values in parenthesis are the mass resolution ($m/\Delta m$) required to separate the analyte ions and interfering species.

^{239}Pu and ^{241}Am) was in the range of 430–520 Mcps mg^{-1} L. It was concluded the advantage of the DIHEN in ICP-MS is the minimization of the solution uptake rate, which is important for the analysis of small amount of sample. The sample solution volume can be reduced even further by the use of nano-volume flow injection (FI) coupled to micronebulizer, allowing the analysis of nanolitre sample volumes in such a way that 100% transport efficiency to the ICP is achieved, and no waste is produced. Using a nano-volume flow injection system and DS-5 nebulizer coupled to ICP-MS, Schaumlöffel et al. [20] achieved detection limits of 10^{-17} mols of U and to 10^{-19} mols of Pu in urine. Only 54 nL of sample was injected into the continuous flow of a solution at 7 μL min^{-1} and introduced into the plasma. In addition to the low detection limits, a relative low UO^+ formation rate was observed, less 2% under optimum operation conditions. The system thus developed is useful for the analysis of small amounts of rare, toxic or radioactive samples.

The aim of the present work was to develop a method for the determination of lanthanides in small volumes of biological samples using a desolvating nebulizer system (APEX) and a nano-volume injection system coupled to a microflow nebulizer (DS-5). The objectives set included (i) reducing the oxides interference (ii) and demonstrating the feasibility of the total consumption nebulizer for lanthanides determination in small amounts of sample.

2. Experimental

2.1. ICP-MS instrumentation

A quadrupole-based ICP-MS (ICP-QMS, ELAN 6100, Perkin-Elmer SCIEX, Concord, Ontario Canada) was used for all mass spectrometric measurements. A high efficiency nebulizer ESI APEX-Q (ESI, Omaha, NE, USA), a microflow total consumption nebulizer DS-5 (CETAC, Omaha, NE) [20] and a MicroMist nebulizer (Model MicroMist AR40-1F02, Glass Expansion, Victoria, Australia) attached to a Cinnabar mini cyclonic spray chamber (Glass Expansion) were used for sample introduction into the ICP-MS plasma. The solutions were introduced into the MicroMist nebulizer by a peristaltic pump (Gilson, France). In the APEX system, the sample was introduced through a PFA microconcentric nebulizer, with a flow intake of 330 μL min^{-1}, working under free aspiration. In this system, the aerosol was introduced into a cyclonic spray chamber heated to 140 °C and then transported to a Peltier-cooled multipass condenser where the temperature was set as −5 °C. In order to avoid any carry-over effect, the APEX system was

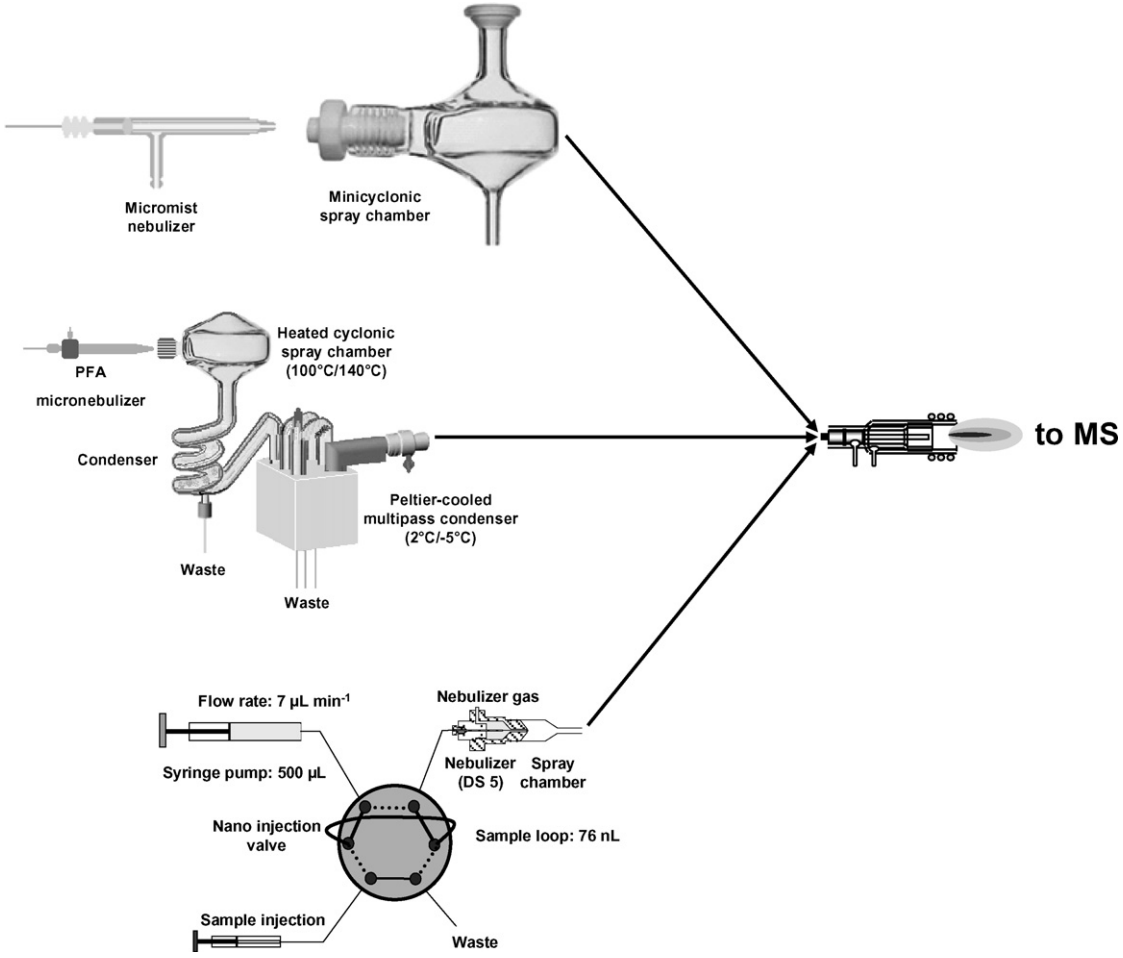

Fig. 1. Schematic of the experimental arrangement for the different systems and nebulizers.

washed out with 1% (v/v) HNO_3 for at least 60 s between each sample. The DS-5 nebulizer was fitted in a low-dead-volume (8 cm^3) single pass spray chamber [20]. A low and constant carrier flow rate of 8 $\mu L\,min^{-1}$ was provided by a high-precision syringe pump (CMA-100, Carnegie Medicine, Solna, Sweden). Nano-volume flow injection was achieved by an ultra-low dead-volume nano-injection valve CN-2 (Valco Instruments, Houston, TX). The sample loop was a 15 cm long and 20 μm i.d. fused silica capillary with an internal volume of 47 nL. Taking into account the internal volume of the injection valve, the total sample volume was 76 nL. The schematic of the experimental arrangement using the different nebulizers is shown in Fig. 1. All measurements were performed under optimized experimental conditions with respect to gas flow rates, rf power, torch position and ion lens voltage, as summarized in Table 2. The lanthanide isotopes shown in Table 2 were monitored by ICP-MS. Except for ^{141}Pr, ^{159}Tb, ^{165}Ho and ^{169}Tm which are monoisotopic lanthanides, in general, the most abundant isotopes of lanthanides free of elemental isobaric interferences (see Table 1) were chosen for mass spectrometric analysis.

2.2. Standards and reagents

All the chemicals used were of Suprapur® grade (Merck, Darmstadt, Germany). Nitric acid was further purified by sub-boiling distillation. All dilutions were made with high-purity deionized water (18 MΩ cm), obtained from a Milli-Q water purified system from Millipore.

To determine the yield of lanthanides oxide and hydrides ion formation in ICP-MS, single element solutions containing 10 $\mu g\,L^{-1}$ were prepared by diluting 1000 $mg\,L^{-1}$ stock solutions (Merck CertiPrep) in 2% (v/v) HNO_3. The calibration was performed with a multielement mixed solution in HNO_3. The acid concentration was 2% (v/v) for the determination in the reference SPS-SW1 water, 15% (v/v) for the organs of the slugs and 7.5% (v/v) for the mussel tissue. External calibration was used throughout. The use of internal standard was not investigated. It would be difficult to have an element to be used as internal standard for lanthanides. This element should be present in negligible concentration in the investigated samples and have properties similar to those of lanthanides. The calibration was performed using at least five calibration solutions. The concentration of the calibration solutions ranged from 0.05 to 2.5 $\mu g\,L^{-1}$ for the APEX system and from 0.25 to 2.5 $\mu g\,L^{-1}$ for the DS-5. A 2% (v/v) HNO_3 solution was used as the sample carrier in the flow injection system.

2.3. Samples and sample preparation

Mussel tissue (Certified Reference Material BCR-668) from the Community Bureau of Reference, surface water level 1, SPS-SW1, from Spectrapure Standards AS (Oslo, Norway), and organs of slugs – genus Arion (gut, salivary gland and oropharynx) were analyzed. Organs were dissected from slug while it was washed with Milli-Q water, which was changed several times during the preparation process. The organs were blotted to superficial dryness with clean absorbing paper towels and then weighted. The mass of the analysed organs were in the range of 28–391 mg. Three 50 mg-aliquots of mussel tissue were microwave-digested (Microwave Accelerated Reaction Systems, MARS-5, CEM Microwave Technology Ltd., USA) using an oxidizing mixture of 0.8 mL HNO_3 and 0.2 mL H_2O_2. The digestion was carried out with the following heating programme: 150 W for 10 min, cooling for 2 min, 300 W for 10 min and cooling for 30 min. The digested sample was transferred to a disposable plastic tube and made up to 10 mL with water. The same procedure was used to digest the organs of the slugs, but in this case the oxidizing mixture was added to the sample 24 h before digestion in the microwave oven. The solution thus obtained was transferred to a disposable plastic tube and the volume made up to 5 mL. Only one replicate was analysed, comprising the entire organ weighed and digested. The SPS-SW1 water sample was acidified to 2% (v/v) HNO_3 just before the analysis, while the concentration of the lanthanides was measured in three different days.

Table 2
Optimized instrumental parameters using a quadrupole based ICP-MS (Elan 6100, Perkin Elmer, Sciex)

	Nebulizer type		
	MicroMist	APEX	DS5
RF power (W)	1250	950	1300
Cooling gas flow rate ($L\,min^{-1}$)	14	14	14
Auxiliary gas flow rate ($L\,min^{-1}$)	1.2	1.2	1.2
Nebulizer gas flow rate ($L\,min^{-1}$)	0.90	0.90	1.0
Solution uptake rate ($\mu L\,min^{-1}$)	700	330	8
Measurement mode	Peak hopping	Peak hopping	Peak hopping
Sweeps/reading	5	5	10–20
Readings/replicate	3	3	40–60
Replicates	3	3	1
Measured isotopes	^{139}La, ^{140}Ce, ^{141}Pr, ^{143}Nd, ^{146}Nd, ^{147}Sm, ^{149}Sm, ^{151}Eu, ^{153}Eu, ^{155}Gd, ^{157}Gd, ^{159}Tb, ^{162}Dy, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{167}Er, ^{169}Tm, ^{172}Yb, ^{173}Yb and ^{175}Lu		
Number of isotopes per run	21	21	1–4
Scanning mode	Peak hopping	Peak hopping	Peak hopping
Sampler and Skimmer cones	Ni	Ni	Ni

3. Results and discussion

3.1. Comparison of performance of micronebulizers used

The argon gas flow rate to the nebulizer and the rf applied have a great effect on ion intensity and the formation of oxide species. Optimization was carried out in terms of nebulizer gas flow rate and rf power to maximize the ion intensities of analytes and minimize the oxide formation rate. The performance of the APEX and DS-5 nebulizer were compared to that of the MicroMist nebulizer coupled to a mini cyclonic spray chamber, which are usually used in routine analysis. The results of optimization procedures are shown for La in Figs. 2 and 3. It can be seen in Fig. 2(a) that the MO^+/M^+ ratio is very low (less than 0.003) for the APEX, irrespective of the gas flow rate. Maximum sensitivity, about 20 Mcps mg^{-1} L, is achieved at a flow rate about 0.7 L min^{-1}, which remains almost the same at higher nebulizer gas flow rates. Compared to other nebulizers, another advantage of the APEX is the higher signal intensity owing to aerosol desolvation, producing an intense and uniform dry aerosol. With respect to the DS-5 nebulizer, in Fig. 2(a) it is observed that the oxides formation is remarkably decreased at nebulizer gas flow rates lower than 1.05 L min^{-1}. However, optimum sensitivity is observed at the range of 1.05–1.50 L min^{-1}. This is a compromise condition, since the flow rate giving the maximum intensity also yields the maximum oxide formation rate. The introduction of aqueous solution at the flow-rates characteristic of the DS-5 nebulizer does not influence the ICP because the introduced solution is evaporated at nebulizer gas flow rates higher than 1.05 L min^{-1} [20]. The gas-phase introduction results in a remarkable plasma stability because of the absence of cold spots induced by larger aerosol droplets. In contrast, with the use of MicroMist (mostly used in routine work) the oxide formation rate increases sharply when the nebulizer gas flow rate increases, while the sensitivity decreases, as shown in Fig. 2(b). For the DS-5, it was observed that the sensitivity measured as a function of the nebulizer gas flow rate is practically constant in the 1.05–1.5 L min^{-1} range after an increase between 0.9 and 1.05 L min^{-1}, which is the same behaviour as observed by Schaumlöffel et al. [20] in the determination of Pu and U.

The study of the influence of rf power on the oxide formation rate, shown in Fig. 3(a), results in a similar behaviour for the MicroMist and the DS-5 nebulizers, but the level of oxides is notably lower for the DS-5 (up to one order of magnitude). Under optimized experimental conditions, the oxide formation rate for the lanthanides was less than 1.5% and 2.5% with the use of DS-5 and MicroMist nebulizers, respectively. For APEX, the oxide formation rate was comparatively low. It ranged from 0.25% to 0.39% at rf power in the range of 950 and 1500 W, whereas the sensitivity was practically constant in this interval (Fig. 3(b)). The sensitivity does not decrease at high rf power for the DS-5, but a

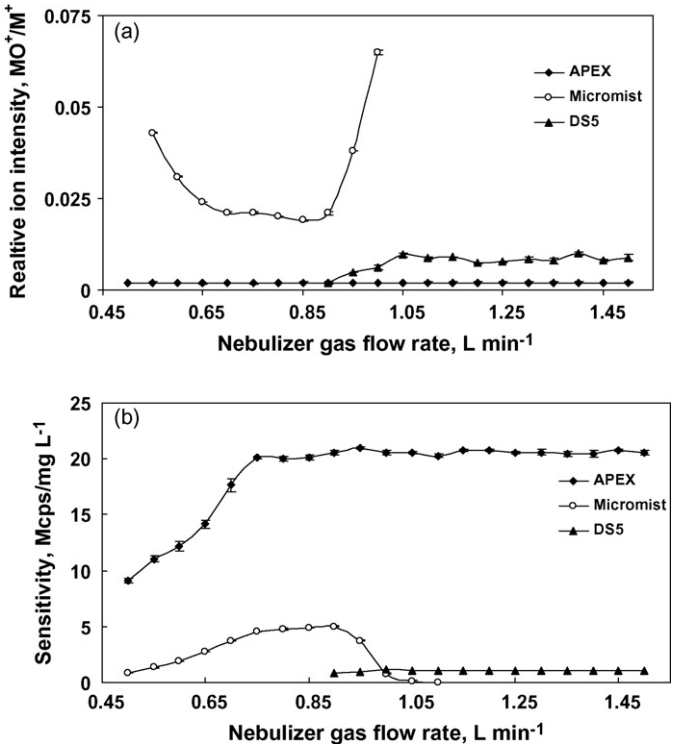

Fig. 2. Effect of nebulizer gas flow rate (rf power: 900 W for the APEX and 1200 W for the MicroMist and DS-5 nebulizers) on the sensitivity and oxide formation rate ($n = 3$). Sample uptake rate: 330 μL min^{-1} for the APEX, 700 μL min^{-1} for the MicroMist and 8 μL min^{-1} for the DS-5 nebulizer. Sample solution: 10 μg L^{-1} La (M^+ and MO^+ represent the intensity of $^{139}La^+$ and $^{139}La^{16}O^+$, respectively).

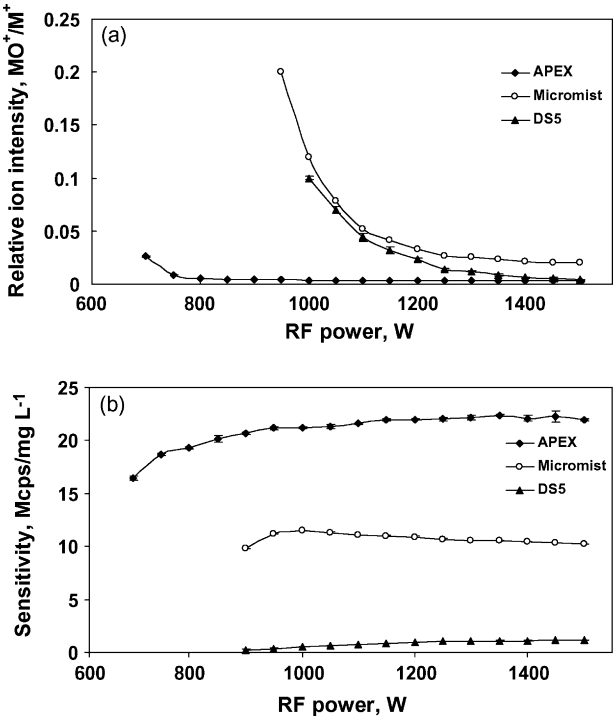

Fig. 3. Effect of the ICP rf power (nebulizer gas flow rate: 0.95 L min^{-1} for the APEX, 1.0 L min^{-1} for the DS-5 and 0.90 L min^{-1} for the MicroMist nebulizers) on the sensitivity and oxide formation rate ($n = 3$). Sample uptake rate: 330 μL min^{-1} for the APEX, 700 μL min^{-1} for the MicroMist and 8 μL min^{-1} for the DS-5 nebulizers. Sample solution: 10 μg L^{-1} La (M^+ and MO^+ represent the intensity of $^{139}La^+$ and $^{139}La^{16}O^+$, respectively).

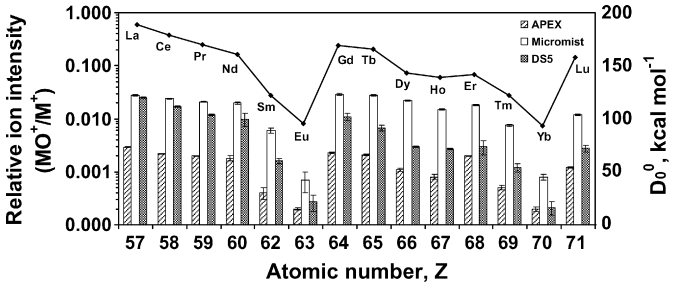

Fig. 4. Relative oxide formation rate of lanthanides using different nebulizers. The upper line refers to the dissociation energy (D_o^0, right *y*-axis) of the element oxide, while the bars are related to the MO^+/M^+ ratio (left *y*-axis).

relatively pronounced decrease is observed for the MicroMist nebulizer.

Regarding the hydride formation rate for lanthanides, the ratio MH^+/M^+ was similar for the three nebulizers, being in the 10^{-4}–10^{-5} range.

To determine low content of lanthanides accurately, possible oxide formation must be considered because oxides of light elements of the series could result in measuring higher ion intensities at $m/z > 155$. As already discussed, in the present work, the lowest oxide formation rate was obtained by using the APEX system. The relative ion intensity (MO^+/M^+) under optimized conditions is compared in Fig. 4 for the three nebulizers investigated. The pattern is similar for the three nebulizers and is in accordance with the oxide dissociation energies (D_o^0) of the lanthanides oxides [21], where a remarkable difference in oxide formation is observed. These findings are also in accordance with a previous work where other nebulizers were used [22]. Lanthanum exhibits the highest MO^+/M^+ value, while Yb exhibits the lower value that correlates with the filling of 4f orbitals [21,22].

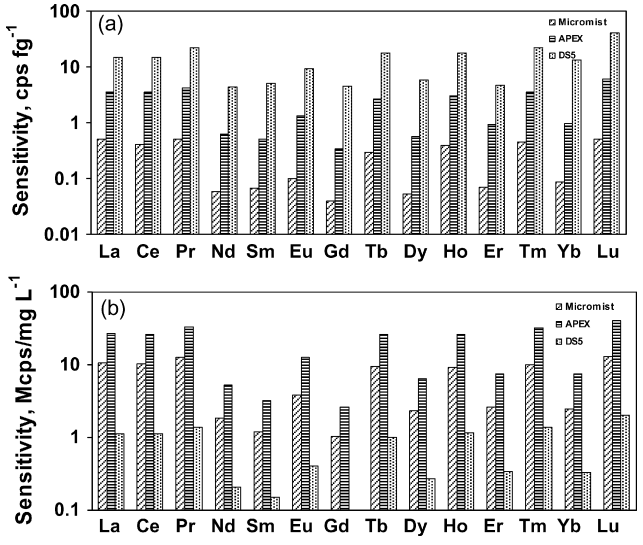

Fig. 5. Sensitivity for lanthanides in ICP-MS using different nebulizers. For the measurements using DS-5, a sample loop of 76 nL was used. The solution uptake rate, in $\mu L\,min^{-1}$, was 330, 700 and 8 (as carrier solution) for the APEX, MicroMist and DS 5 nebulizers, respectively.

Table 3
Relative (in $ng\,L^{-1}$) and absolute (in pg or fg) detection limits of lanthanides measured with the different nebulizers at the conditions shown in Table 1

Isotope	Limit of detection					
	APEX		MicroMist		DS-5	
	$ng\,L^{-1}$	pg	$ng\,L^{-1}$	ng	$ng\,L^{-1}$	fg
^{139}La	1.8	21	4.2	0.10	55	4.2
^{140}Ce	1.4	16	3.8	0.095	80	6.1
^{141}Pr	1.0	12	3.0	0.075	40	3.1
^{146}Nd	5.0	54	18	0.46	165	12
^{152}Sm	1.6	19	16	0.41	90	6.8
^{153}Eu	2.2	25	11	0.28	77	5.9
^{156}Gd	6.1	71	13	0.33	95	7.2
^{159}Tb	0.57	6.7	3.5	0.087	60	4.6
^{163}Dy	3.2	45	11	0.28	100	7.6
^{164}Ho	2.1	24	3.6	0.090	65	4.9
^{166}Er	3.5	41	12	0.29	140	11
^{169}Tm	1.8	21	3.3	0.082	70	5.3
^{172}Yb	3.7	43	9.9	0.25	170	13
^{175}Lu	0.85	9.9	2.9	0.072	30	2.3

3.2. Sensitivity and detection limits

An important parameter for comparing different small sample volume nebulizers is the absolute sensitivity (number of counts per femtogram of analyte) and relative sensitivity ($Mcps/mgL^{-1}$) obtained with the same ICP mass spectrometer [23]. Fig. 5 shows the sensitivity of the investigated nebulizers with respect to lanthanides. The better relative sensitivity observed for APEX in Fig. 5(a) is due to aerosol desolvation, which not only improves aerosol quality, but also increases transport efficiency in comparison to the MicroMist nebulizer. The quality of aerosol, which may negatively affect the ionization efficiency and thus the sensitivity, and the lower sample transport efficiency are the possible reasons for the poorer sensitivity of the MicroMist nebulizer. The lowest relative sensitivity for the DS-5 is, obviously, due to the low solution uptake rate. On the other hand, the high absolute sensitivity obtained for the same nebulizer in Fig. 5(b) is explained by the high transport efficiency (total consumption).

The relative and absolute limits of detection (LODs) of analytes measured with the different nebulizers are summarized in Table 3. The LODs were obtained from $3s$, being s the standard deviation of 10 consecutive measurements of the blank. The lowest absolute detection limits are obtained with the DS-5 nebulizer. For monoisotopic lanthanides such as Tb and Lu the LODs were at sub ppt level. The lowest relative detection limits are obtained in ICP-MS with the APEX nebulizer with desolvator. In general, the results demonstrate the possibility of measuring very low concentrations of lanthanides in small volume of samples. The DS-5 nebulizer detection limits were calculated by monitoring a single isotope per run.

3.3. Validation of the developed ICP-MS method

For method validation, lanthanides were determined in the SPS-WS1 reference water. As shown in Table 4, the con-

Table 4
Average concentrations and standard deviations ($n=6$), in $\mu g\,L^{-1}$, of lanthanides found in reference water SPS-SW1 (informed value: $0.5\,\mu g\,L^{-1}$)

Element	Nebulizer	
	APEX	DS-5
La	0.59 ± 0.02	0.60 ± 0.05
Lu	0.46 ± 0.02	0.53 ± 0.05
Ce	0.54 ± 0.02	0.57 ± 0.08
Pr	0.55 ± 0.03	0.61 ± 0.04
Nd	0.52 ± 0.03	0.59 ± 0.12
Sm	0.55 ± 0.02	0.48 ± 0.07
Eu	0.53 ± 0.02	0.66 ± 0.13
Gd	0.57 ± 0.04	0.62 ± 0.11
Tb	0.48 ± 0.04	0.59 ± 0.06
Dy	0.78 ± 0.05	0.64 ± 0.09
Ho	0.53 ± 0.03	0.54 ± 0.04
Er	0.53 ± 0.03	0.67 ± 0.10
Tm	0.52 ± 0.03	0.53 ± 0.08
Yb	0.51 ± 0.02	0.50 ± 0.03

centrations measured agree with the information value of $0.5\,\mu g\,L^{-1}$, with the exception of Dy. Higher Dy concentration than the informed value was found using both the APEX and the DS-5 nebulizer. In general, the results obtained with the use of the APEX combined with ICP-QMS are more precise, which can be seen from the lower standard deviations. According to Fig. 4, this behaviour was already expected since the oxides formation rate (MO^+/M^+) is higher using the DS-5 nebulizer. The concentration of Ba in the SPS-SW1 water is 100-fold higher than those of lanthanides ($50{,}000\,ng\,L^{-1}$) and BaO^+ may interfere on the Nd^+, Eu^+, Sm^+ and Gd^+ ion signals (see Table 1). In addition, the oxides of lighter lanthanides may interfere on the heavier lanthanides (Table 1). This may be the reason for the relative high standard deviation of Er, measured using the DS-5 nebulizer.

Table 5
Lanthanides concentration (average and standard deviation), $ng\,g^{-1}$, measured in mussel tissue (BCR 668) using the APEX system for introducing the solution of the digested sample into the ICP-MS plasma ($n=3$)

Element	Certified	Found
La	80 ± 6	85 ± 2
Lu	0.389 ± 0.024	0.363 ± 0.043
Ce	89 ± 7	85 ± 4
Pr	12.3 ± 1.0	12.1 ± 0.9
Nd	54 ± 4	50 ± 1
Sm	11.2 ± 0.8	12.3 ± 0.6
Eu	2.79 ± 0.15	3.02 ± 0.36
Gd	13.0 ± 0.6	12.0 ± 0.7
Tb	1.62 ± 0.11	1.44 ± 0.18
Dy	8.9 ± 0.6	7.9 ± 1.0
Ho[a]	1.8 ± 0.6	1.6 ± 0.3
Er	4.47 ± 0.45	3.78 ± 0.52
Tm	0.48 ± 0.08	0.38 ± 0.03
Yb[a]	2.8 ± 0.5	2.7 ± 0.02

[a] Indicative values.

Table 6
Lanthanides concentration, $ng\,g^{-1}$, found in organs of slugs using the DS-5 nebulizer for introducing the solution of digested sample into the ICP-MS plasma ($n=3$)

Element	Gut	Salivary Gland	Oropharynx
La	636 ± 20	40.0 ± 2.2	3376 ± 234
Lu	<3.9	<0.39	7385 ± 311
Ce	1163 ± 25	69.1 ± 2.5	699 ± 64
Pr	123 ± 10	9.6 ± 0.7	1610 ± 171
Nd	590 ± 56	106 ± 11	285 ± 27
Sm	117 ± 23	20.7 ± 2.5	61.5 ± 8.4
Eu	6.9 ± 0.5	11.0 ± 1.9	432 ± 30
Gd	114 ± 27	18.7 ± 2.5	54.9 ± 5.1
Tb	6.76 ± 1.3	<0.77	188 ± 9
Dy	133 ± 16	<1.3	41.8 ± 5.2
Ho	<8.5	2.30 ± 0.43	214 ± 17
Er	<18	<1.8	14.3 ± 2.1
Tm	<9.1	<0.91	182 ± 8
Yb	<22	<2.2	21.7 ± 0.6

3.4. Determination of lanthanides in small biological tissues

To validate and apply the developed method it was employed for lanthanides determination at the $ng\,g^{-1}$ level in mussel tissue and three selected slug organs, respectively. The concentrations measured in the certified reference mussel tissue (BCR 668) by ICP-MS are compared with the certified values in Table 5. The concentrations found by ICP-MS are well within the 95% confidence level of the certificate or close to the indicative values, which demonstrates the feasibility of the APEX as a solution introduction system in ICP-MS for lanthanides determination in biological samples. The DS-5 micronebulizer was not utilized for this sample, since the concentrations of most analytes would be lower than their limits of detection (see Table 3). Therefore, the DS-5 micronebulizer was applied for the analysis of different slugs organs, where the sample amount available was very low (several mg only). The concentrations in the three different slugs organs measured by ICP-MS are summarized in Table 6. Generally we observed a gradient for all lanthanides with highest concentrations (up to 7.4 and $3.4\,\mu g\,g^{-1}$ for Ce and La, respectively) in the oropharynx, intermediate concentrations in the gut and lowest in the salivatory gland. Our findings would be compatible with the majority of lanthanide species being bioavailable and subdued to renal elimination. For non-absorbable species comparable concentrations would be expected in the oropharynx and the gut. Furthermore, an enrichment of lanthanides in the chitin rich masticating apparatus composed of radula and odontophore as part of the oropharynx has to be examined. We can exclude an enrichment of lanthanides in the salivatory gland. In general, lower concentrations were observed for elements with odd atomic numbers compared to elements with neighboring even atomic numbers. This lanthanide distribution in biological tissues with higher concentrations for lighter lanthanides compared to heavier ones correlated qualitatively with the distribution in nature (e.g., in geological samples). The relative standard deviation (RSD) of mass spectrometric measurements was mostly less than 10%. The relatively good

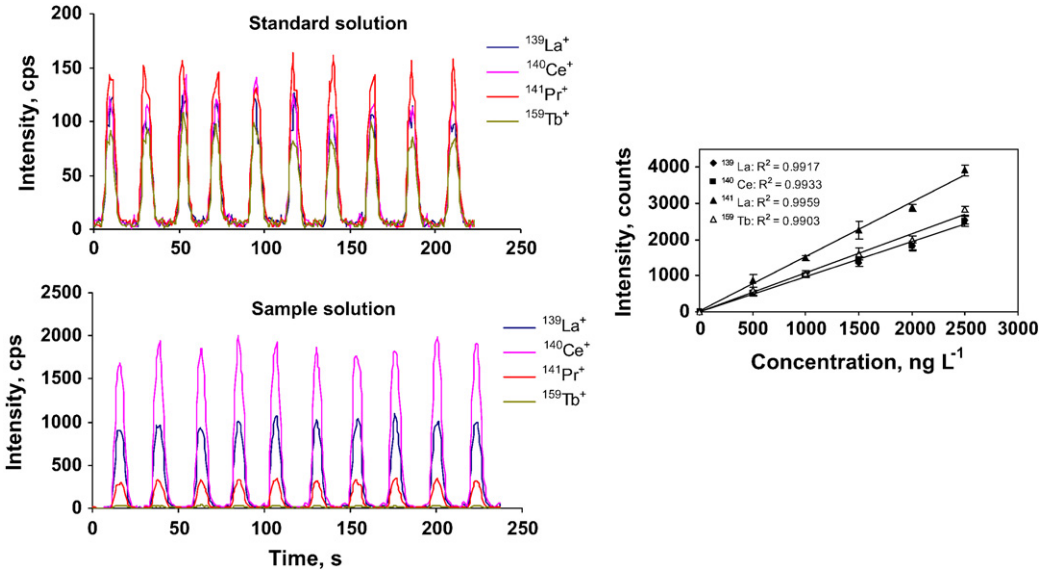

Fig. 6. Peak profiles for 10 repeated measurements of 500 ng L^{-1} La, Ce, Pr and Tb solution (standard) and in salivatory gland of slug (sample). The insert corresponds to the calibration curves.

precision can also be observed for the results presented in Fig. 6, being the RSD for 10 consecutive measurements 7.5%. Calibration curves like those shown in the insert of Fig. 6 were typically obtained by using the DS-5 and four isotopes per run measured. The correlation coefficients of the calibration curves were determined with values better than 0.99. In Fig. 6, the transient signals of ICP-MS measurements of 500 ng L^{-1} La, Ce, Pr and Tb in standard solution and in salivatory gland of slug sample are illustrated. The signal shape is similar for the analytes in the standard and sample solutions, which means there is no physical matrix interference. Considering the very small sample volume analyzed (76 nL), the performance of nano-volume FI system is feasible for lanthanides determination in biological samples.

4. Conclusion

This study demonstrated that the application of the microneb-ulizer with desolvator (APEX), which drastically reduces oxide formation, coupled to a quadrupole ICP mass spectrometer is advantageous for the determination of lanthanides at low concentration levels in environmental and biological samples. The very small sample consumption indicates the potential of the used nano-volume flow injection system coupled to a microneb-ulizer (DS-5) for the determination of lanthanides when the available amount of sample is very low. However, although oxide formation is minimized with the use of DS-5, possible oxides interference cannot be excluded, especially if the concentration of Ba is relatively high in the sample. Under optimized experimental conditions, the hydride formation rate of lanthanides with the aid of both nebulizers is typically in the 10^{-4}–10^{-5} range. Therefore, with the use of the investigated nebulizers, interference by hydrides is not relevant in the determination of lanthanides in the biological samples investigated. The analytical methods developed were successfully utilized for lanthanide determination at the ng g^{-1} level in small amounts of biological tissue by ICP-MS after microwave digestion.

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