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Article

Determination of Hg and Se in Biological Materials by Chemical Vapor Generation Electrothermal Vaporization Inductively Coupled Plasma Mass Spectrometry using Isotope Dilution Calibration after Microwave-Assisted Digestion with *Aqua Regia*

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Propõe-se um método para a determinação de Hg e Se em materiais biológicos por espectrometria de massa com plasma indutivamente acoplado e introdução da amostra por vaporização eletrotérmica após geração química de vapor (CVG-ETV-ICP-MS) e calibração por diluição isotópica. As amostras foram digeridas com água régia em um sistema convencional de microondas. As razões isotópicas usadas para quantificação foram: $^{201}Hg/^{202}Hg$ e $^{77}Se/^{82}Se$. Borohidreto de sódio foi usado como agente redutor. As temperaturas de retenção e vaporização foram de 150 e 2000 °C, respectivamente. Seis materiais biológicos certificados foram analisados e as concentrações obtidas para Hg e Se foram concordantes com os valores certificados de acordo com o teste-*t* para um nível de confiança de 95%. Os limites de detecção obtidos foram 0,7 e 3 ng g^{-1} para Hg e Se, respectivamente. O método é preciso, exato e adequado para a análise de materiais biológicos em rotina e demonstra a viabilidade do uso da diluição isotópica no sistema proposto.

A method for the determination of Hg and Se in biological materials by chemical vapor generation electrothermal vaporization inductively coupled plasma mass spectrometry (CVG-ETV-ICP-MS) using isotope dilution calibration after acid digestion is proposed. The samples were digested with *aqua regia* in a microwave oven. The isotope ratios used for quantification were: $^{201}Hg/^{202}Hg$ and $^{77}Se/^{82}Se$. A $NaBH_4$ solution stabilized with NaOH was used as reducing agent. The retention and vaporization temperatures in the graphite tube were 150 and 2000 ºC, respectively. Six certified biological materials were analyzed and the obtained concentrations were in good agreement with the certified values according to the *t*-test for a confidence level of 95%. The detection limits in the sample were 0.7 and 3 ng g^{-1}, for Hg and Se respectively. The method is precise, accurate and adequate for the analysis of biological samples in routine and demonstrates the feasibility of using isotope dilution for the proposed system.

Keywords: chemical vapor generation, biological samples, microwave digestion, retention on the graphite tube, isotopic dilution calibration, ETV-ICP-MS

Introduction

The quality of the analytical results mainly depends on the sample pre-treatment stages and on the detection system, principally for determination of elements with low concentration in the samples, such as Hg and Se. The most common way to solubilize biological sample is the microwave-assisted acid digestion with a concentrated acid, usually HNO_3, $HClO_4$ and HF and H_2O_2 or mixtures of them in a closed vessel.[1] Other interesting alternative is the use of slurry sampling that combines the advantages of the liquid and solid sampling presentations, with a simplified sample preparation. However, the use of slurry sampling for biological samples in the determination of Se by chemical vapor generation (CVG) can be difficult, because of the presence of its organic species, such as Se–cystine, Se–methionine and trimethylselenonium, that need to be broken before the hydride generation and because the resulting oxidation state must be adequate for CVG.[2-4] Santos *et al.*[5] have evaluated 5 different procedures for the slurry preparation concerning the determination of Hg and Se in biological samples by axial

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view inductively coupled plasma optical emission spectrometry (ICP-OES) using on-line CVG, concluding that the procedures that utilize either H_2O_2 or $K_2S_2O_8$ and sonication, before heating with HCl, are adequate for the Se determination, confirming that the organic molecules must be efficiently broken, before CVG. It is reported that the hexavalent oxidation state of Se does not generate a measurable signal, after hydride generation, demonstrating that it has to be converted to Se(IV) before CVG.[6] Boiling HCl in the concentration range of 5-6 mol L^{-1}, for at least 15 min, is almost exclusively used for this purpose.[7] The microwave-assisted sample preparation is used for a wide range of applications, including decomposition of inorganic and organic materials.[8] Brisbin and Caruso[9] evaluated different procedures using microwave extraction for the determination of As, Cd, Co, Mo and Se in lobster tissue.

Among the analytical techniques employed to analyze biological material, inductively coupled plasma mass spectrometry (ICP-MS) is especially important due to its several advantages, such as its multielement capacity, high sensitivity and ability to measure isotopic ratios, which allows isotope dilution calibration (ID).[10] Electrothermal vaporization (ETV) is also currently used in ICP-MS to vaporize and introduce the sample vapor into the plasma.[11-13] The isotope dilution (ID), as a calibration procedure for ICP-MS, has being employed for elements having more than one isotope. The technique of ID requires the measurement of the altered isotopic ratio, after the addition of an enriched isotope. Isotopic dilution is considered to be the ideal calibration, as the isotope of an element is an ideal internal standard for its own determination, being considered the procedure that produces the most accurate results by ICP-MS. However, absence of spectral interferences and equilibration between the added enriched isotope and the isotope in the sample are required conditions. By using this calibration technique, the non-spectral interferences and analytes losses after equilibration of the added enriched isotope are compensated.[14-15]

The isotope dilution calibration was previously employed in electrothermal vaporization coupled to inductively coupled plasma mass spectrometry (ETV-ICP-MS). Vanhaecke *et al.*[14] reported the use of isotope dilution calibration for solid sampling by ETV-ICP-MS. Chang and Jiang[16] applied the ETV-ICP-MS for the determination of Cu, Cd and Pb in several biological samples using isotopic dilution calibration. The feasibility of using isotope dilution calibration ETV-ICP-MS to analyze samples prepared as slurries was investigated by Maia *et al.*[17] for the determination of Cd, Hg, Pb and Tl in coal and coal fly ash slurries.

Retention of the vapor produced by CVG on a treated-graphite tube is also an interesting possibility for the analysis by electrothermal atomization atomic absorption spectrometry (ET-AAS) or by ETV-ICP-MS. Vieira *et al.*[18] proposed the determination of As, Hg, Se and Sn in sediment slurries, after chemical vapor generation and retention of the vapor on an Ir-treated graphite tube heated at 150 °C of an ETV-ICP-MS system, using external calibration. After retention, the analytes were released by heating the vaporizer cell to 2000 °C and transported to the plasma by an argon flow. Recently, Vieira *et al.*[19] have investigated the possibility of using the isotope dilution calibration for the determination of Cd, Hg, Pb and Se in sediments slurries by CVG-ETV-ICP-MS, with and without retention of the vapor on the graphite tube. They observed that the excellent results obtained with the isotope dilution calibration should be attributed to an efficient extraction of the analyte to the aqueous phase of the slurry, leading to the equilibration of the added isotopes with the isotopes of the sample, which is a basic requirement for the ID calibration. Retention of the vapor on the graphite tube allows *in situ* preconcentration and elimination of kinetic effects during the vapor generation and vapor transport from the solution to the plasma.

In this work, a method for the determination of Hg and Se in biological samples, after microwave-assisted acid digestion with *aqua regia* of the samples followed by chemical vapor generation with retention of the analyte vapor in an Ir-treated graphite tube of an electrothermal vaporizer using isotope dilution calibration (ID-CVG-ETV-ICP-MS) is optimized and described. According to our knowledge, this procedure was not proposed previously.

Experimental

Instrumental

All measurements were carried out with an inductively coupled plasma mass spectrometer ELAN 6000 (Perkin Elmer SCIEX, Thornhill, ON, Canada). For the ETV system, a hydride generator MHS-15 (Perkin Elmer, CO, USA) was coupled to a Perkin-Elmer HGA 600 MS electrothermal vaporizer and a Perkin Elmer AS-60 autosampler and manually operated, as described previously.[18]

A 3% (m/v) sodium borohydride solution stabilized with 1% (m/v) sodium hydroxide was used as reducing agent in the MHS-15. The reducing agent was injected during 5 s using an argon pressure of 250 kPa. The generated vapors were transported to the ETV by an Ar flow through a 10 cm glass tube (0.1 cm i.d.) connected

to a polytetrafluorethylene (PTFE) tube (60 cm long, 0.5 cm i.d.). Pyrolytic coated graphite tubes (Perkin-Elmer, Part No. B050 8371) were used. Argon of 99.996% purity (White Martins, São Paulo, Brazil) was used. An internal Ar flow rate of 0.1 L min^{-1} in the vaporizer, optimized previously,[18] summed to the "nebulizer" gas flow rate of 1.06 L min^{-1} during vaporization, resulting in a total flow rate of 1.16 L min^{-1}, transported the aerosol to the plasma.

The instrumental conditions are shown in Table 1 and the ETV temperature program, which also was optimized previously,[18] is shown in Table 2. The samples for the microwave digestion were weighed using a M2P microbalance (Sartorius, Göttingen, Germany). A Milestone (Sorisole, Italy) Model Ethos Plus microwave system was employed for the digestions of the samples.

Table 1. ETV-ICP-MS operational parameters

Parameters	
RF power / (W)	1000
Gas flow rate / (L min^{-1})	
Principal	15
Intermediate	1.2
Carrier	1.06
Sampler and skimmer cones	Pt
Dwell time / (ms)	25
Sweeps *per* reading	1
Reading *per* replicate	150
Resolution at 10% of the peak height	0.7 u.m.a
Auto lens	On
Signal measurement	Peak area

Table 2. ETV temperature program

Step	Temperature / (°C)	Ramp / (s)	Hold / (s)	Gas flow rate / (mL min^{-1})
Cooling	20	1	5	300
Pre-heating[a]	150	5	0	0
Pre-heating[b]	150	1	30	0
Cooling	20	1	8	0
Vaporization[c]	2000	1	20	100
Cleaning	2200	5	5	300
Cooling	20	1	5	300

[a]glass tube introduction into the graphite tube; [b]analyte vapor collections on the graphite tube; [c]vaporization and reading.

Reagents and materials

All reagents were of analytical grade. The water (resistivity of 18.2 MΩ cm) used was de-ionized in a Milli-Q system (Millipore, Bedford, MA, USA). Nitric acid (Carlo Erba, Milan, Italy, No. 408015) and hydrochloric acid (Merck, Darmstadt, Germany, No. K27703017) were further purified by sub-boiling distillation in a quartz still (Kürner Analysentechnik, Rosenheim, Germany). The reducing agent was prepared by dissolving $NaBH_4$ (Merck, No. K25622271) in NaOH (Merck, No. B665769) stored in a polyethylene flask and kept under refrigeration for no longer than two days. A stock solution of $IrCl_3$ (Fluka, Buchs, Switzerland, No. 58195), 1000 mg L^{-1} Ir, was used for the treatment of the tube with the permanent modifier as described previously.[20] The enriched isotope materials were from the Cambridge Isotope Laboratories Inc. (Andover, MA, USA). The abundances of the enriched isotopes were: 96.35% of ^{201}Hg and 93.48% of ^{77}Se. The isotope compositions of the enriched materials were measured and the found values agreed with the informed values. Stock solutions of 30 mg L^{-1} for Hg and 200 µg L^{-1} for Se were prepared by dissolution of an accurately weighed amount of the solid material (HgO and elemental Se) in nitric acid and diluted in 5% v/v HNO_3. The following isotope ratios were used in the calculation of the concentrations: $^{201}Hg/^{202}Hg$ and $^{77}Se/^{82}Se$. The ratio of the signal intensities of the isotopes in the samples without the addition of the enriched isotope was compared to the natural ratio in order to check for spectral interference and mass discrimination. The correction factor for the mass discrimination was calculated by comparing the natural isotope ratio with the measured ratio for the sample without the spike. The software automatically uses the correction factor to correct the measured altered ratio.

The following certified reference materials were used: BCR 397 (human hair) and BCR 186 (lyophilized pig kidney) from the Community Bureau of Reference (Brussels, Belgium), DOLT-2 and DOLT-3 (dogfish liver), DORM-2 (dogfish muscle) and TORT-2 (lobster hepatopancreas) from the National Research Council of Canada (Ottawa, Ontario, Canada).

Sample preparation

An aliquot of approximately 250 mg of the sample was weighed directly in the PTFE flask of the microwave oven. Then, the solutions of the materials enriched with the isotopes ^{201}Hg and ^{77}Se were added to the flask in an adequate amount in order to obtain an altered ratio close to 1, to minimize the measurement errors. Due to the low concentrations of Se and Hg in the samples, masses of the enriched materials at ng level were added. The masses were calculated using the ID equation for an altered ratio of 1 and converting the masses to volumes of the enriched materials solutions. The sample aliquot without spike was digested with 3.5 mL of *aqua regia*

plus 1.0 mL of deionized water in the microwave flask. The sample aliquot with the spikes was also digested with 3.5 mL of *aqua regia* plus a certain volume of deionized water, which was the complement to 1 mL of the volumes of the spiking solutions. In this way, all sample aliquots, without and with spikes, were digested in the same volume of liquid. The samples were then submitted to a 5-steps microwave oven temperature program (2 min at 50 °C and 400 W maximum power, 2 min at 50 °C and 200 W maximum power, 6 min at 80 °C and 550 W maximum power, 5 min at 80 °C and 1000 W maximum power, 7 min at 160 °C and 1000 W maximum power). After the digestion was finished, all solutions were colorless, indicating an effective digestion. Following that, 1 mL of the digested sample was mixed with 1 mL of concentrated HCl and heated to 90 °C for 30 min, in order to guarantee the lower oxidation state for Se, favoring its hydride generation.[7] After cooling the solution, the final volume of 10 mL was made up with deionized water, which was not strictly necessary, as isotopic dilution calibration was used, assuming that equilibration of the added isotope with the isotope in the sample have occurred during digestion. However, this final dilution to the same final volume for each sample aliquot, allowed a better control of the counting signals.

Analytical procedure

For the determination of Hg and Se in biological samples, a 1 mL aliquot of the final sample solution (without or with spike), was transferred to the reaction flask of the hydride generator and the temperature program of the ETV was started. The glass tube of the hydride generator was manually introduced into the graphite tube. During the pre-heating step, the reducing agent was added to the reaction flask for 5 s and the generated vapors were transferred to the graphite tube by using argon for 30 s in the MHS-15. Before the vaporization step, the glass tube was removed and the graphite tip of the ETV arm closed the dosing hole of the graphite tube. Reading was carried out during the vaporization stage, in which the vapor is transported from the ETV to the plasma by a total argon flow rate of 1.16 L min^{-1}.

Results and Discussion

The chemical vapor generation conditions and the electrothermal vaporizer temperature program were optimized previously for the determination of Hg and Se and other analytes in sediments, as slurries in an *aqua regia* plus HF medium, using external[18] or isotopic dilution calibration.[19] The isotopes ^{202}Hg (natural abundance of 29.8%) and ^{82}Se (natural abundance of 8.73%) were chosen as reference isotopes, taking into account the absence of spectral interference and their natural abundances. In this way, the concentrations were obtained by measuring simultaneously the following altered isotope ratios: ^{201}Hg/^{202}Hg and ^{77}Se/^{82}Se.

Effect of the HCl treatment and of the aqua regia concentration

In order to generate the vapor, the sample should be completely digested, meaning that all organic compounds containing the analytes should be destroyed. The microwave-assisted digestion of the biological sample with *aqua regia* efficiently digested the biological samples, as clear solutions were obtained. As already mentioned, the isotope dilution is an ideal internal standardization, as the internal standard, in this case, is one isotope of the same element. In principle, if a reasonable fraction of the Se, after the digestion, was present in its lower oxidation state, which is able to generate the hydride, the isotope calibration should compensate for the fraction of the Se, which is on its higher oxidation state and does not generate the hydride.[6] In this way, a procedure was attempted, in which the sample solution, after digestion, was not heated with HCl, but was only taken to a final volume of 10 mL with 1 mol L^{-1} HCl. The obtained concentrations for Se were significantly higher (from 19% to 50%) than the certified values, indicating that the heating with HCl, which converts Se(VI) to Se(IV) is required even when isotope dilution calibration is employed, what could indicate that the Se(IV) fractions in the spike and in the sample are different. However, for Hg, the obtained results, with and without heating with HCl, are in agreement with the certified values according to the *t*-test for a 95% confidence level. Thus, 1 mL of the sample solution was mixed with 1 mL of HCl and heated to 90 °C for 30 min after the sample digestion and before the vapor generation.

The *aqua regia* volume added to the sample mass of around 250 mg for the microwave-assisted digestion was investigated, using the DOLT-2 sample. A volume of 3.5 mL of *aqua regia* was used. Lower volumes do not lead to accurate results neither for Hg nor for Se, being the obtained concentrations much lower than the certified ones, indicating that part of the analytes are not in the appropriate forms to generate the cold vapor

of Hg or the hydride of Se. Most probably, the lower volume is not enough to mineralize completely the sample.

Figures of merit and analytical application

The limit of detection (LOD) is defined as the minimum concentration or weight of analyte that can be detected at a known confidence level. The limits of detection, in the sample, calculated as a function of the enrichment of the isotopic spike and the linear calibration detection limits for each isotope[21] are 0.7 and 3 ng g^{-1} for Hg and Se, respectively, indicating that the proposed procedure is able to detect very low concentrations of the analytes in biological samples.

In a very recent paper, Santos *et al.*[5] have investigated the chemical vapor generation for Hg and Se in biological samples as slurries in different media, prior to detection by in ICP-OES, using external calibration. For 20 mg of solid sample in 15 mL of slurry, the obtained detection limits (3 s, n = 10) for the proposed procedure were 80 and 100 ng g^{-1} for Hg and Se, respectively, much higher (about 110 times for Hg and 33 times for Se) than those obtained in this work, as expected due to the higher sensitivity of the ETV-ICP-MS technique. Vieira *et al.*[18] have proposed a method for the determination of As, Hg, Se and Sn in sediment by slurry sampling CVG-ETV-ICP-MS, also with trapping of the vapor on a treated graphite tube, but using slurry sampling and external calibration against aqueous standards, obtaining about the same LOD (3 s, n = 10) for Hg, 0.80 ng g^{-1}, as in this work, and 300 times better LOD for Se, 0.01 ng g^{-1}.

In Figure 1 some typical transient signals for Hg and Se in reference material DOLT-2, before and after addition of the enriched isotopes, are shown. As shown in the Figure 1, the condition of an altered isotopic ratio close to 1 was obtained for Hg and Se.

The accuracies of the procedures were estimated by the analysis of six certified reference materials. As shown in Table 3, the found concentrations are in agreement with the certified values, according to the *t*-test for a confidence level of 95%. The relative standard deviations (RSD) were in the range from 0.4 to 9.4% for Se and from 1.8 to 14.3%, for Hg, indicating an adequate precision. Probably, an efficient digestion of the of the sample, with complete destruction of the analytes organic compounds by microwave digestion with *aqua regia* was attained, providing the equilibration of the added isotopes with the isotopes in the sample, which is a basic requirement for the ID calibration. After equilibration, there is no need to use an exact volume of the sample solution.

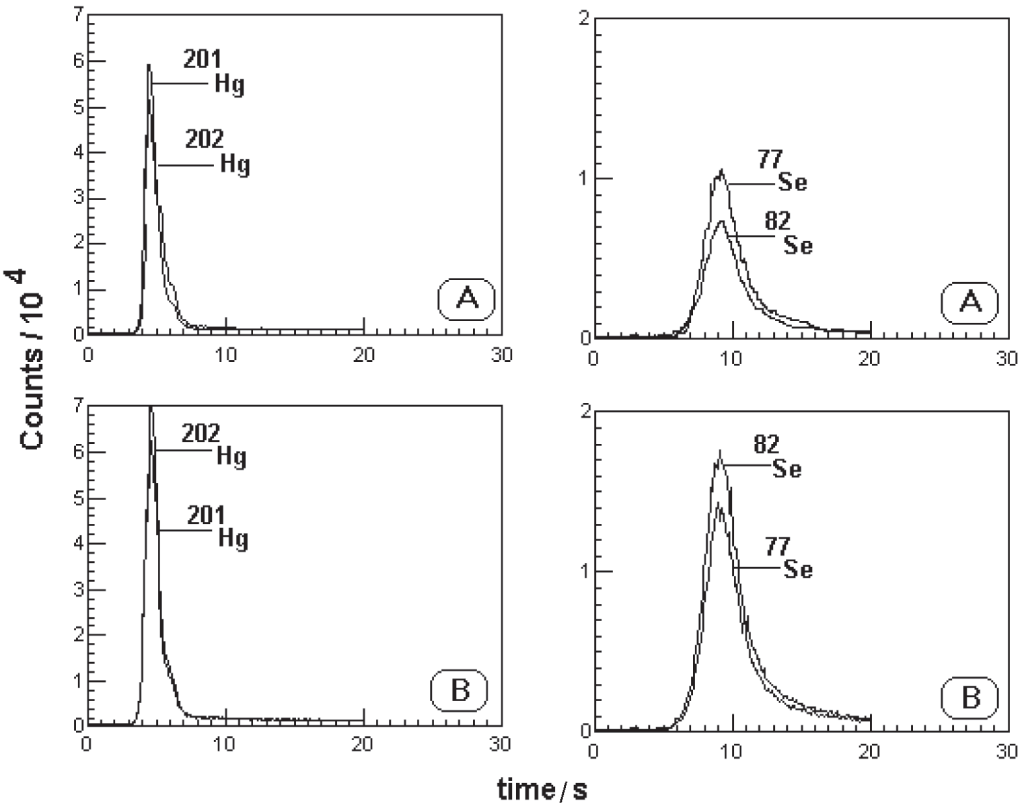

Figure 1. Transient signals for Hg and Se in reference material DOLT-2 (a) without and (b) with the addition of the enriched isotope.

Table 3. Determined concentrations in μg g^{-1} for Hg and Se in certified biological materials by CVG-ETV-ICP-MS using isotope dilution calibration after microwave digestion with *aqua regia* and treatment with HCl to reduce the oxidation number of Se (n = 5)

Sample	Hg	Se
DOLT-2		
Certified	2.14 ± 0.28	6.06 ± 0.49
Found	2.33 ± 0.06	5.94 ± 0.31
RSD (%)	2.6	5.2
DOLT-3		
Certified	3.37 ± 0.14	7.06 ± 0.48
Found	3.27 ± 0.07	7.63 ± 0.18
RSD (%)	2.1	2.3
DORM-2		
Certified	4.64 ± 0.26	1.40 ± 0.09
Found	4.34 ± 0.08	1.27 ± 0.12
RSD (%)	1.8	9.4
BCR 186		
Certified	1.97 ± 0.04	10.3 ± 0.5
Found	1.89 ± 0.09	9.9 ± 0.04
RSD (%)	4.8	0.4
BCR 397		
Certified	12.3 ± 0.5	2.00 ± 0.08
Found	11.9 ± 1.7	1.96 ± 0.12
RSD (%)	14.3	6.1
TORT-2		
Certified	0.27 ± 0.06	5.63 ± 0.67
Found	0.24 ± 0.03	5.61 ± 0.27
RSD (%)	12.5	4.8

Conclusions

It was demonstrated that Hg and Se can be determined in biological samples after microwave-assisted acid digestion with *aqua regia* followed by their determination by CVG-ETV-ICP-MS using vapor trapping in the graphite tube and isotopic dilution calibration. The treated tube with Ir was efficient for the trapping of the Hg cold vapor and the Se hydride. The proposed procedure using ID compensate eventual analyte loss during the steps of sample preparation, including digestion and reduction of the oxidation state for Se. The reduction of the oxidation state for Se with HCl prior to its hydride generation is necessary, even using isotope dilution calibration. For Hg, the reduction step with HCl can be omitted.

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