

Determination of sulfur in biological samples using high-resolution molecular absorption spectrometry in a graphite furnace with direct solid sampling

Hadla S. Ferreira,^a Fábio G. Lepri,^a Bernhard Welz,^{*ab} Eduardo Carasek^a and Mao-Dong Huang^c

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The determination of sulfur in biological materials using high-resolution continuum source molecular absorption spectrometry and electrothermal vaporization of the carbon monosulfide (CS) molecule has been investigated in detail using direct solid sampling. Best results have been obtained coating the platform with tungsten as a permanent modifier, adding 40 μg Pd in solution as a chemical modifier, and the only sulfur compound that showed sufficient sensitivity and thermal stability to be used for calibration purposes under the conditions established for biological materials was thiourea. A pyrolysis temperature of at least 900 °C could be used and the optimum vaporization temperature was 2500 °C. Under optimized conditions a limit of detection of 0.015 μg S absolute or 0.03 mg g^{-1} S in the solid sample, based on a sample mass of 0.5 mg could be obtained; the characteristic mass was $m_0 = 18$ ng. Five certified biological reference materials have been analyzed using direct solid sampling and calibration against aqueous standards; the results were in agreement with the certified values on a 95% confidence interval.

1. Introduction

Sulfur is with a content of about 0.048% the 15th most abundant element of our earth's crust; it can be found in elemental form in huge volcanic deposits, mainly in Italy, North America and Japan. The most important minerals are copper, iron, lead and zinc sulfide and sodium and alkaline earth sulfates. Sulfur is an essential element for plants and animals; it makes part of various amino acids, co-enzymes and vitamins. The gaseous compounds H_2S and SO_2 , however, are highly toxic; the latter one is liberated in great quantities by combustion of fossil fuel, and it has contributed significantly to acid rain.

Sulfur is determined by a variety of techniques, including gravimetric^{1,2} and volumetric methods,³ UV-Vis spectrophotometry,⁴⁻⁹ ion chromatography,¹⁰ X-ray fluorescence,¹¹ inductively coupled plasma mass spectrometry (ICP-MS)^{12,13} and inductively coupled plasma optical emission spectrometry (ICP OES).¹⁴ UV-Vis spectrophotometry is among the most widely used techniques for the determination of sulfur, but requires extensive sample preparation and is subject to various interferences from other ions. UV fluorescence measurement of SO_2 after combustion of the sample at temperatures >1000 °C appears to be a reliable and sensitive technique for the determination of sulfur in a variety of materials;¹⁵ however, special equipment is required for this analysis.

The sensitivity of the determination by ICP-MS is affected by the high ionization potential of 10.36 eV, and polyatomic interferences due to $^{16}\text{O}_2^+$, $^{16}\text{O}_2\text{H}^+$, $^{16}\text{O}^{18}\text{O}^+$, and $^{36}\text{Ar}^+$ are a frequent problem.

High-resolution ICP-MS,¹³ the use of reaction/collision cells,¹⁶ thermal¹⁷ or electrothermal vaporization^{18,19} can solve these problems and result in very low limits of detection (LOD), however, at the expense of increased cost and complexity. ICP OES may be used as well for the determination of sulfur, but the most sensitive emission lines are all in the vacuum UV, requiring special equipment.

The first attempts to determine sulfur by molecular absorption spectrometry (MAS) date back to the early 1970s, when Syty²⁰ and Winkler and Syty²¹ investigated the absorption bands of SO_2 , studies that were later extended to the H_2S molecule by Cresser and Isaacson.²² These investigations were carried out using a kind of chemical vapor generation at room temperature in a reaction flask and MAS of a cold gas. Dittrich and Vorberg²³ were the first to report a determination of sulfur *via* MAS of the GeS molecule using electrothermal vaporization (ETV) in a graphite furnace (GF) and a H_2 hollow cathode lamp (HCL) as the radiation source. A dual-channel atomic absorption spectrometer was used for the measurements and background correction was carried out using the two-line method.

Tittarelli and Lavorato²⁴ determined sulfur in fuel oils by MAS of the carbon monosulfide (CS) molecule at 257.6 nm generated by ETV in a GF using a deuterium (D_2) lamp and reported a LOD of 50 mg kg^{-1} in the undiluted oil. Parvinen and Lajunen²⁵ used ETV in a GF to investigate the determination of sulfur *via* the molecular absorption of AIS, InS and SnS. Best results were obtained measuring SnS molecular absorption, which has strong absorption bands between 271 and 316 nm and between 418 and 451 nm. Using the emission line of tungsten HCL at 273.6 nm the authors could achieve a LOD of 1 mg L^{-1} sulfur. One of the major challenges was to avoid losses of sulfur during the drying and pyrolysis stages and the authors investigated various chemical modifiers.

The major problem with all these approaches was the relatively low resolution of the spectrometers, which could only be

^aDepartamento de Química, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, SC, Brazil. E-mail: w.bernardo@terra.com.br; Fax: +55 48 3721 6850; Tel: +55 48 3733 8876

^bInstituto Nacional de Ciência e Tecnologia do CNPq-INCT de Energia e Ambiente, Universidade Federal da Bahia, 40170-115 Salvador, BA, Brazil

^cLeibniz-Institut für Analytische Wissenschaften-ISAAS-e.V., Department Berlin, 12489 Berlin, Germany

overcome using line sources, *i.e.*, HCL of elements that had by chance an emission line that coincided with a molecular absorption 'line' of the molecule of interest. Another problem was the background, as the deuterium lamp background correction used by most of the authors is not designed to deal with any structured background, which is typical for the absorption spectra of diatomic molecules.²⁶ The situation changes completely when equipment for high-resolution continuum source AAS is used for MAS; this technique and its special features are fully described in the monograph of Welz *et al.*²⁷

Huang *et al.*²⁸ reported the determination of total sulfur in wine in an air-acetylene flame using a rotational line at 258.056 nm, which is part of the CS molecular band system with the band head at 257.595 nm. In order to produce a sufficiently high concentration of CS it was necessary to use an extremely fuel-rich flame. In later work²⁹ the same authors investigated the determination of S *via* MAS of CS in more detail; they recorded the spectra of 17 elements and found that there is no risk for spectral interference at the 258.056 nm line. They also investigated 12 cations and four mineral acids for potential non-spectral interference; only lead at a concentration above 200 mg L⁻¹ caused a reduction of the CS absorption due to precipitation of lead sulfate from the solution. The authors reported a LOD of 2.4 mg L⁻¹ sulfur. Huang *et al.*³⁰ also proposed an interesting speciation analysis of the different sulfur forms in wine, *i.e.*, free SO₂, total SO₂, bound SO₂, sulfate and total sulfur. The method is based on the finding that the sensitivity for 'free SO₂' in slightly acidified solution is much higher than that for all other sulfur forms. This is due to the fact that the free SO₂ is almost quantitatively transferred into the vapor phase upon nebulization in the burner chamber, whereas 90–95% of the sulfur compounds in solution are going down the drain in the aerosol conditioning process.

Heitmann *et al.*³¹ investigated the determination of sulfur *via* the MAS of CS in a GF using a Zr-coated graphite tube. In order to obtain an efficient formation of CS the authors found it necessary to add methane (4% v/v) to the argon purge gas in the pyrolysis stage. They used calcium as a modifier, which made possible a pyrolysis temperature of 700 °C. The authors reported a characteristic mass of $m_0 = 12$ ng for the strongest line at 258.056 nm, a LOD of 2.3 ng sulfur and analyzed two plant reference materials.

The goal of the present work has been to investigate in more detail the determination of sulfur in biological materials using high-resolution graphite furnace MAS (HR-GF MAS) of the CS molecule. Special emphasis has been put on the optimization of the analytical conditions, including the use of modifiers and appropriate calibration solutions. The final goal has been to investigate the feasibility of direct analysis of solid samples using aqueous standards for calibration, an approach that was found to be particularly successful using high-resolution continuum source GF AAS.³²

2. Experimental

2.1 Instrumentation

A prototype high-resolution continuum source atomic absorption spectrometer, built at ISAS, Berlin, has been used for most

of the measurements in this work. The equipment is based on a Model AAS 6 Vario (Analytik Jena AG, Jena, Germany) from which all optical components, including the detector and associated controls have been removed and replaced by a high-resolution continuum source spectrometer similar to that described by Becker-Ross *et al.*³³ This spectrometer consists of a high-intensity xenon short-arc lamp operating in a hot-spot mode, a high-resolution double monochromator with a resolution of $\lambda/\Delta\lambda \approx 140,000$ (corresponding to about 2 pm per pixel), and a charge coupled device (CCD) array detector. Details of this equipment have been described previously.^{27,34} The peak volume selected absorbance (PVSA),³⁵ *i.e.*, the integrated absorbance summed over three pixels around the line core ($A_{\Sigma 3, \text{int}}$) has been used for signal evaluation, unless stated differently, as the best signal-to-noise (S/N) ratio has been obtained under these conditions. Some measurements have been carried out using a Model contraAA 700 high-resolution continuum source atomic absorption spectrometer (Analytik Jena, Jena, Germany), which uses the same radiation source and a similar optical system, only with a slightly higher resolution of $\lambda/\Delta\lambda \approx 175,000$.

The conventional transversely heated graphite tube atomizer system supplied by Analytik Jena together with the Models AAS 6 Vario and contraAA 700 has been used throughout. Most of the experiments have been carried out using pyrolytically coated SS graphite tubes without dosing hole (Analytik Jena Part No. 407-A81.303) and SS platforms (Analytik Jena Part No. 407-152.023). Pyrolytically coated PIN-platform tubes (Analytik Jena Part No. 407-A81.025) and graphite tubes without pyrolytic coating (Analytik Jena Part No. 407-A81.022) have been used for some of the experiments. Solid samples were weighed directly onto the SS platforms using an M2P microbalance (Sartorius, Göttingen, Germany, accuracy 0.001 mg) and inserted into the graphite tube using a pre-adjusted pair of tweezers, which is part of the SSA 5 manual solid sampling accessory (Analytik Jena). Aqueous standards were injected manually onto the SS platform using micropipettes with disposable tips. Argon (99.996%, White Martins, São Paulo, Brazil) was used as purge and protective gas. A mixture of 4% methane in argon (Linde, São Paulo, Brazil) has been used as alternate gas in the pyrolysis stage for some of the experiments.

2.2 Standards, reagents and samples

All reagents were at least of analytical grade (p.a.). Nitric acid was further purified by sub-boiling distillation in a quartz apparatus (Kürner Analysentechnik, Rosenheim, Germany). De-ionized water from a Milli-Q system (Millipore, Bedford, MA, USA) with a resistivity of 18 MΩ cm has been used throughout. Stock standard solutions containing 1000 mg L⁻¹ sulfur have been prepared by dissolution of ammonium sulfate, magnesium sulfate, sulfuric acid, sodium sulfite, thiourea, sulfanilic acid and sulfanilamide (all from Merck, Darmstadt, Germany) in deionized water. Calibration solutions have been prepared by appropriate dilution of the stock standard solutions with deionized water.

Palladium (10 g L⁻¹) in 15% v/v nitric acid, magnesium nitrate (Merck), calcium carbonate (Riedel-de-Haen), a zirconium standard solution (Johnson Matthey, Karlsruhe, Germany) and

tungsten oxalate (SPEX, Edison, NJ, USA) in 5% v/v nitric acid have been used as modifiers.

The following certified reference materials (CRM) have been used: NIST RM 8433 Corn Bran, NIST SRM 1568a Rice Flour, NIST SRM 1549 Non-fat Milk Powder, (National Institute of Standards and Technology, Gaithersburg, MD, USA), NCS DC 73349 Bush Branches and Leaves and NCS ZC 73014 Tea–trace elements, (National Analysis Center for Iron and Steel, Beijing, China). All CRM have been used ‘as supplied’ without any further treatment.

2.3 Procedure

Tungsten has been used as permanent modifier for most of the experiments; the modifier was thermally deposited onto the platform surface by 10 repetitive injections of 30 μL of a 2 g L^{-1} W stock solution. After each injection, the temperature program shown in Table 1 has been applied. Aliquots containing between 0.1 and 1.0 mg of samples were weighed directly onto the SS platforms and inserted into the graphite tube with the manual SSA 5 accessory. Calibration (10 μL) and modifier (20 μL) solutions were deposited manually onto the SS platforms using a micropipette with disposable tips. The temperature program adopted for aqueous standards (calibration) and for solid samples is shown in Table 2.

3. Results and discussion

3.1 The high-resolution CS molecular absorption spectrum

The integrated absorbance spectrum of the CS molecule around 258.0 nm, which is part of the $\Delta\nu = 0$ vibrational sequence of the electronic transition $X^1\Sigma^+ \rightarrow A^1\Pi$, where the strongest rotational ‘lines’ are observed, is shown in Fig. 1a. The line at 258.033 nm has been chosen for all measurements because of a slight spectral interference due to high iron concentrations at the 258.056-nm line proposed by others,^{29,31} as shown in Fig. 1b. Initially the analytical conditions used by Heitmann *et al.*³¹ have been adopted, except for coating the platforms with tungsten as

Table 1 Temperature program for the deposition of tungsten on the SS graphite platform; Ar flow-rate 2.0 L min^{-1} in all stages

Step	$T/^\circ\text{C}$	Ramp/ $^\circ\text{C s}^{-1}$	Hold time/s
1	90	10	20
2	120	5	20
3	400	50	20
4	1000	100	10
5	2000	100	5

Table 2 Temperature program for the determination of sulfur *via* CS in biological materials by solid sampling HR-GF MAS

Stage	$T/^\circ\text{C}$	Ramp/ $^\circ\text{C s}^{-1}$	Hold time/s	Ar flow rate/ L min^{-1}
Drying 1	90	10	10	2.0
Drying 2	120	5	15	2.0
Pyrolysis	900	50	15	2.0
Vaporization	2500	3000	5	0
Cleaning	2650	1000	5	2.0

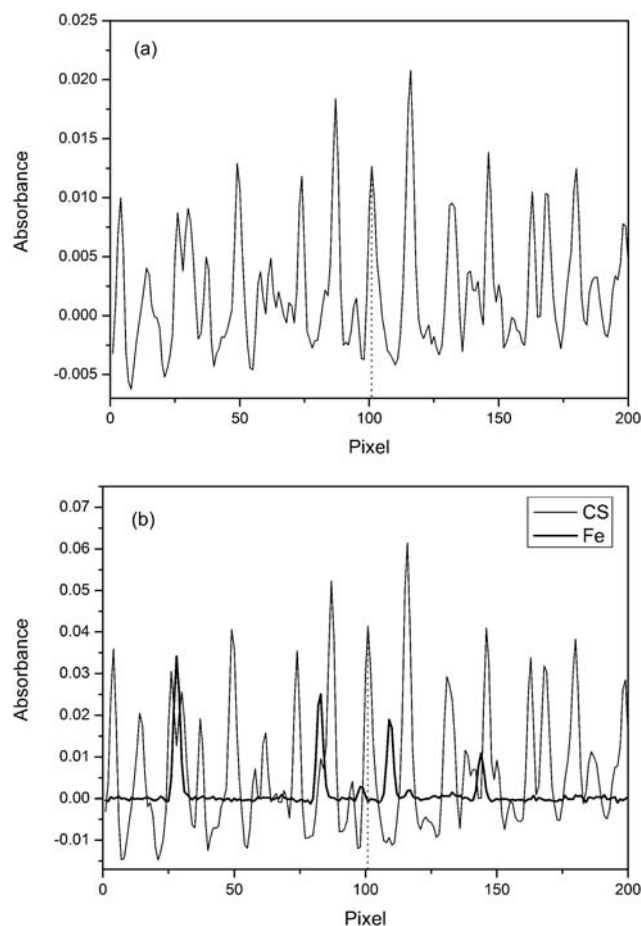


Fig. 1 Absorbance spectrum of the CS molecule in the range between 257.890 nm and 258.175 nm using 40 μg Pd as chemical modifier and the conditions of Table 2; the analytical line at 258.033 nm is marked with a dotted line. (a) 1.0 μg S as thiourea in aqueous solution; (b) NCS DC 73349 Bush Branches and Leaves CRM with the addition of 20 μg Fe.

permanent chemical modifier instead of zirconium. All other conditions have been the same, *i.e.*, ammonium sulfate has been used to prepare the standard solutions, calcium was used as the modifier and a mixture of 4% methane in argon has been used as an alternate gas in the pyrolysis stage.

It has to be kept in mind that in GF MAS, in contrast to GF AAS, two analytical conditions have to be considered to obtain optimum results. Firstly, the analyte has to be stabilized to sufficiently high pyrolysis temperatures in order to remove the majority of the matrix without losses of the analyte element—which is the same as in GF AAS. Secondly, the formation of the target molecule has to be promoted in order to obtain maximum sensitivity; this is significantly different from the task to promote analyte atomization, which usually only includes the choice of an optimum atomization temperature.

3.2 Evaluation and comparison of modifiers

3.2.1 Aqueous standard solutions. First of all, coating of the platform with refractory carbide-forming elements, such as Ta, Ti, W and Zr, as permanent modifier was investigated. All of these modifiers clearly had a positive effect on the formation of

CS in the graphite furnace, and Zr resulted in the best sensitivity, which is in agreement with the results of Heitmann *et al.*³¹ Tungsten coating produced a very similar sensitivity but showed a slightly better long-term stability; hence the SS platforms were coated with tungsten for most of the future measurements, which resulted in a threefold increase in sensitivity compared to a pyrolytically coated platform. The mechanism in which tungsten and the other refractory carbide-forming elements act to increase the sensitivity is not fully understood yet; however, the classical procedure to synthesize CS₂ by conducting sulfur vapor over charcoal at 800–1000 °C,³⁶ might give an indication. Sulfur that is in direct contact with graphite might be intercalated, which could result in the formation of CS₂ at higher temperatures, instead of the target molecule CS. Due to its extremely high volatility, any CS₂ would most likely be lost immediately upon its formation. Tungsten coating obviously reduces the contact with the graphite surface in the condensed phase significantly, avoiding intercalation and the formation of CS₂.

As a next step, the 4% methane in argon mixture, used by Heitmann *et al.*³¹ in the pyrolysis stage, was investigated to obtain an efficient formation of CS. The experiments were carried with an aqueous standard of (NH₄)₂SO₄ and the NIST RM 8433, using flow rates of 0.5, 0.7 and 2.0 L min⁻¹ of the mixture, respectively. The results obtained for both, the aqueous standard and the CRM, did not indicate any significant increase in the absorbance signal measured for CS, unless a very long pyrolysis time was used. The use of graphite tubes without pyrolytic graphite coating (using platforms coated with WC) has also been tested in order to promote formation of CS due to a gas-solid reaction with the tube surface and to reduce the oxygen content in the gas phase; however, no significant increase in the absorbance of CS has been observed either. For this reason, all future experiments were carried out without the 4% methane in argon mixture and using pyrolytically coated graphite tubes only.

In addition to the tungsten permanent modifier, Ca, Mg and Pd, respectively, were studied as chemical modifiers added in solution to aqueous standards prepared from (NH₄)₂SO₄. The optimum modifier mass was found to be around 40 µg for all

three modifiers. All investigated modifiers had a significant effect on the absorbance signal obtained for sulfur in aqueous standards, demonstrating that the addition of a modifier in solution in addition to the permanent modifier is essential to obtain good sensitivity. The use of Ca as the modifier resulted in the best sensitivity, which is in agreement with the results of Heitmann *et al.*,³¹ and the enhancement effect of Mg was similar. The sensitivity obtained with the Pd modifier, in contrast, was only about half of that obtained with Ca.

3.2.2 Biological reference materials. However, this situation changed significantly when the biological CRM were investigated. Fig. 2 shows the effect of the Ca and Pd modifiers on the thermal stability and sensitivity obtained for two CRM in comparison to the situation without using a modifier. Without modifier losses of sulfur start already at pyrolysis temperatures around 400 °C and the sensitivity is low. Calcium as a chemical modifier could stabilize the analyte to higher pyrolysis temperatures; however, the improvement in sensitivity was marginal and was not reproducible over time. In addition, the integrated absorbance obtained for the CRM was not proportional to the certified sulfur content and not comparable to that obtained for aqueous standards, indicating a strong matrix influence and serious calibration problems.

Good sensitivity has usually been obtained with new graphite tubes, but the tubes and platforms were deteriorating rapidly, resulting also in a loss of sensitivity. It is well known that calcium forms a carbide CaC₂ with a melting point around 2300 °C, which reacts with water under the formation of acetylene. This could well be the reason for the rapid deterioration of the graphite parts and the sensitivity, as any calcium carbide that remains on the platform after atomization will react with the aqueous modifier solution introduced for the next determination, volatilizing part of the graphite material in the form of acetylene.

In contrast to this, palladium not only stabilized sulfur in the CRM up to about 1600 °C, but it also provided the highest sensitivity, and there was no deterioration of this performance over time. For these reasons a mass of 40 µg of Pd has been chosen as the modifier for all future investigations.

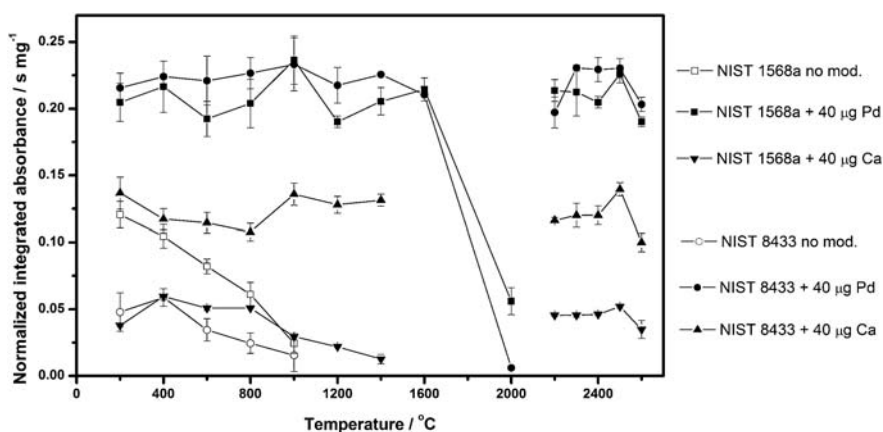


Fig. 2 Pyrolysis and atomization curves for two CRM without modifier, with 40 µg Ca and 40 µg Pd, respectively, as the chemical modifier; NIST SRM 1568a Rice Flour — □ — without modifier; — ■ — with 40 µg Pd and — ▼ — with 40 µg Ca as the chemical modifier; NIST RM 8433 Corn Bran — ○ — without modifier; — ● — with 40 µg Pd and — ▲ — with 40 µg Ca as the chemical modifier.

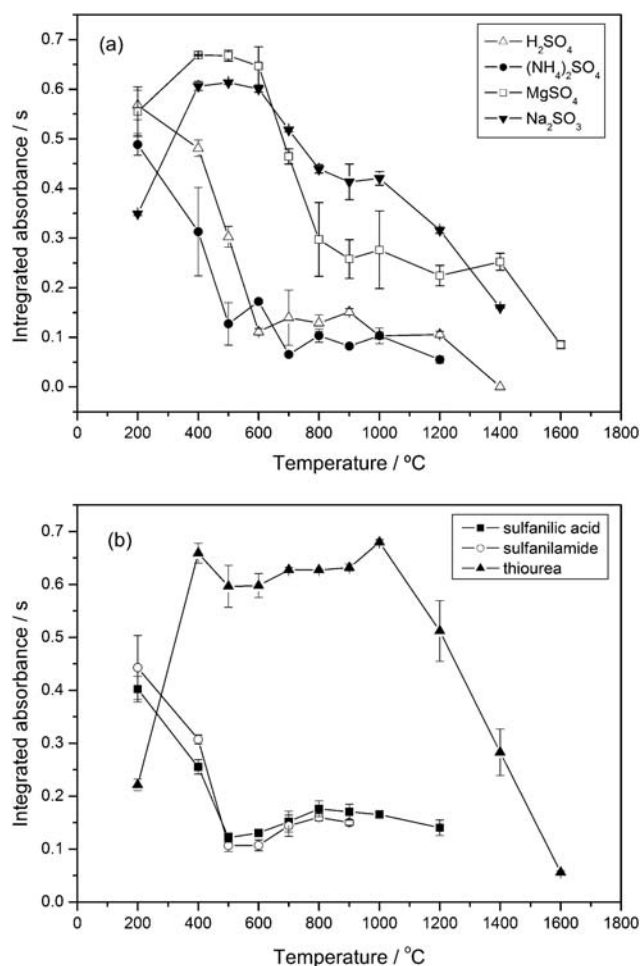


Fig. 3 Pyrolysis curves for aqueous sulfur standards containing $2.5 \mu\text{g S}$, using $40 \mu\text{g Pd}$ as the chemical modifier; (a) inorganic sulfur compounds; (b) organic sulfur compounds.

3.3 Standard solutions for calibration

While the absorbance signal for sulfur in the CRM (in the form of CS molecular absorption) could be controlled quite easily using the palladium modifier, the aqueous calibration standards have been a real challenge. Fig. 3a shows the thermal behavior of four inorganic sulfur standards in the presence of $40 \mu\text{g}$ of Pd as the modifier. Both, the ammonium sulfate recommended by Heitmann *et al.*,³¹ and sulfuric acid could not be stabilized at all, losing sensitivity at pyrolysis temperatures above 200°C . Magnesium sulfate and sodium sulfite could at least be stabilized up to a pyrolysis temperature of about 600°C , which, however, was unsatisfactory compared to the pyrolysis temperatures that could be reached for the CRM.

Palladium was used successfully by others as a modifier for the determination of sulfur using ETV-ICP-MS¹⁹ and ETV-ICP OES,³⁷ respectively. Nevertheless, sulfur could be stabilized only to pyrolysis temperatures of 400°C and 600°C , respectively. In the case of ETV-ICP-MS¹⁹ this low stabilizing power might be due to the low mass of only $0.5 \mu\text{g Pd}$ used as a modifier; however, in the case of ETV-ICP OES³⁷ this explanation cannot be used, as these authors used $10 \mu\text{g Pd}$, a value that is close to that used in our work. In addition it has to be pointed out that in

the ICP-based techniques any sulfur compound that reaches the plasma will be atomized and ionized and hence detected, whereas in HR-GF MAS only the target molecule CS can be detected. Mroczek *et al.*¹⁹ found that $10 \mu\text{g Pd}$ could stabilize $(\text{NH}_4)_2\text{SO}_4$, Na_2SO_4 and H_2SO_4 to a similar extent up to 600°C and proposed that the stabilization is due to a palladium sulfide Pd_xS_y . Resano *et al.*¹⁹ found that a palladium signal always appears together with the sulfur signal, which supports the formation of such a compound. However, both authors did not use any refractory carbide coating, which might explain the differences with the results found in this work, as in their case sulfur might be additionally stabilized by intercalation and later be volatilized as CS_2 , as discussed previously.

As the investigated CRM obviously contained a significant amount of carbon it has been suspected that the lack of carbon in the standard solutions might be responsible for the low sensitivity and thermal instability. Several organic sulfur compounds have therefore been investigated as potential candidates to be used for calibration. As shown in Fig. 3b, only one of these compounds, thiourea, provided satisfactory results, both with respect to sensitivity and thermal stability up to 1000°C . This standard has therefore been chosen for calibration in all future experiments, as it also provided sensitivity very close to that obtained for the CRM. A pyrolysis temperature of 900°C was used for aqueous standards and the CRM, as the organic matrix could be removed easily at this temperature.

3.4 Background and spectral interference

One of the features of high-resolution continuum source AAS is the automatic correction for spectral events that are considered 'continuous', *i.e.*, which affect the radiation flux at all pixels at the same time and to the same extent.²⁷ The same system is obviously active in MAS, and it corrects for any fluctuation in the emission intensity of the continuous radiation source and for continuous background absorption. After automatic correction for continuous spectral events remain visible all spectrally

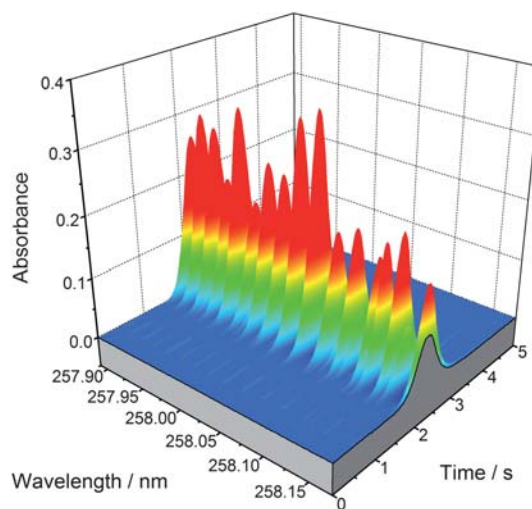


Fig. 4 Time- and wavelength-resolved absorbance spectrum obtained for NCS DC 73349 Bush Branches and Leaves CRM under optimized conditions.

discontinuous events, such as atomic absorption and molecular absorption with rotational fine structure, like the absorption of the CS molecule used in this work for the determination of sulfur. Atomic absorption lines could cause spectral interference in the determination of sulfur in case they are overlapping with the rotational line of the CS molecule used for this purpose.

Huang *et al.*²⁹ investigated the spectra of 16 potentially interfering elements, and found that only iron had two absorption lines at 258.006 nm and 258.045 nm that could theoretically interfere with the determination of sulfur at 258.033 nm. The potential interference of iron has already been shown in Fig. 1b, and the 258.033-nm line has been used in this work instead of the 258.056-nm line proposed by others.^{29,31} Fig. 4 shows the time- and wavelength-resolved absorbance spectrum obtained for the NCS DC 73349 'Bush Branches and Leaves' CRM under optimized conditions. There is no other concomitant absorbance visible besides the CS molecular spectrum, and the baseline exhibits an extremely low noise level.

3.5 Figures of merit

The main analytical parameters of the developed procedure, such as sensitivity, limit of detection and quantification and precision are summarized in Table 3. The limit of detection (LOD) has been calculated according to the "zero-mass response" as three times the standard deviation of the signal obtained for 10 repetitive 'atomizations' of a tungsten-coated SS platform only

Table 3 Figures of merit for the determination of sulfur in biological materials *via* CS molecular absorption using SS-HR-CS GF MAS, W (600 µg) as permanent chemical modifier and Pd (40 µg) added in solution. Calibration with aqueous standards prepared from thiourea; pyrolysis temperature 900 °C; vaporization temperature 2500 °C

Parameter	Value
Inclinations µg ⁻¹	0.241 ± 0.012
Calibration range/µg	0.05–3.00
<i>R</i>	0.999
LOD/µg	0.015
LOQ/µg	0.05
^a LOD/mg g ⁻¹	0.03
^a LOQ/mg g ⁻¹	0.10
Characteristic mass, <i>m</i> ₀ /ng	18

^a Calculated for 0.50 mg of sample.

Table 4 Results obtained for the determination of sulfur in biological CRM *via* CS molecular absorption using SS-HR-CS GF MAS with W (600 µg) as permanent modifier and Pd (40 µg) added in solution. Calibration with aqueous standards prepared from thiourea; pyrolysis temperature 900 °C; vaporization temperature 2500 °C; all values in mg g⁻¹ (*n* = 5)

CRM	Certified	Found ^a
NIST RM 8433 Corn Bran	0.86 ± 0.15	0.83 ± 0.03
NCS DC 73349 Bush Branches and Leaves	7.3 ± 0.6	6.6 ± 0.5
NCS ZC 73014 Tea	3.0 ± 0.3	3.3 ± 0.2
NIST SRM 1568a Rice Flour	1.20 ± 0.02	1.15 ± 0.11
NIST SRM 1549 Milk Powder	3.51 ± 0.05	3.51 ± 0.38

^a All results expressed as mean and confidence interval at 95% level.

containing the palladium modifier,³⁸ divided by the slope of the working curve. The limit of quantification (LOQ) is based on the same measurements, using ten times the standard deviation of the blank. LOD and LOQ have also been established for the solid samples taking into account a typical sample mass of 0.50 mg used in the proposed method. The characteristic mass of *m*₀ = 18 ng is in reasonably good agreement with the value of 12 ng reported by Heitmann *et al.*³¹ for the slightly more sensitive wavelength at 258.056 nm. The precision expressed as relative standard deviation (%RSD) for 10 replicate analyses of a sample was usually around 10%, which is typical for the direct analysis of solid samples due to their natural heterogeneity.

3.6 Analysis of CRM

The measurements of sulfur *via* CS for SS-HR-GF MAS by the proposed method were carried out in five CRM with different matrices, and the result obtained for the determination are presented in Table 4. The values obtained for the five samples were consistent with the certificates with 95% confidence, demonstrating the good accuracy of the proposed method using aqueous standards for calibration. The fact Heitmann *et al.*³¹ found accurate results for two plant CRM using the Ca modifier and (NH₄)₂SO₄ for calibration – a method that has been found not suitable in this work – is probably due to the digestion procedure used in this work, which converts all sulfur into sulfate. In addition the authors used a 'three-point addition procedure' for calibration, which obviously compensated for matrix effects, in case there were any.

4. Conclusion

The results demonstrated that the direct determination of sulfur *via* CS molecular absorption in biological materials by HR-GF MAS is feasible and that the method has the potential for routine analysis. The investigations have shown that it is easier to find an appropriate modifier for the analysis of biological materials than to find a suitable standard for calibration. Palladium has been found to be efficient not only to prevent analyte losses in the pyrolysis stage up to at least 900 °C, but also to significantly improve the sensitivity of the CS absorption in the samples and in the thiourea standards. It might be expected that sulfur could be determined in other kind of samples using similar procedures.

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