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Determination of sulfur in bovine serum albumin and L-cysteine using high-resolution continuum source molecular absorption spectrometry of the CS molecule☆

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ABSTRACT

In this study, the content of sulfur in bovine serum albumin and L-cysteine was determined using high-resolution continuum source molecular absorption spectrometry of the CS molecule, generated in a reducing air-acetylene flame. Flame conditions (height above the burner, measurement time) were optimized using a 3.0% (v/v) sulfuric acid solution. A microwave lab station (Ethos Plus MW) was used for the digestion of both compounds. During the digestion step, sulfur was converted to sulfate previous to the determination. Good repeatability (4–10%) and analytical recovery (91–106%) was obtained.

1. Introduction

High-resolution continuum source atomic and molecular absorption spectrometers are commonly employed for sequential multi-element determination using the atomic lines, but they can also be applied to determining elements such us phosphorus, fluorine or sulfur using the fine structure of the molecular absorption bands. Molecular absorption spectrometry with a high-resolution continuum source atomic and molecular spectrometer has been commonly used in the last decade for the determination of non-metals, specially sulfur, in a wide range of applications using flames or furnaces [1–5].

Resano et al. [6] have critically examined the potential of the technique for the analysis of metalloids and non-metals. Sulfur has been determined in coal samples [7], petroleum products [8,9], and different types of foods and beverages [10–12] using the CS absorption rotational lines [13]. The SnS molecule has also been employed to determine the element using a graphite furnace [14].

Some of the traditional methods for the determination of proteins include the measurement of absorption at 205 or 280 nm using bovine serum albumin (BSA) as the standard, the Biuret reaction, the Lorry method based on the use of the Folin-Ciocalteau reagent, the Kjeldahl method based on the measurement of N, dyeing with the Comassie reagent (Bradford method), and dyeing with silver nitrate or fluorescamine. The selection of the method depends on the type of sample, amount of protein, interferences and cost of analysis. The working range for the measurement of UV absorption at 280 nm is 50 mg L^{-1}–2 g L^{-1} for the Bradford method and 1 mg L^{-1}–1.5 g L^{-1} for the Lorry method. The limits of detection can decrease to 10 μg L^{-1} with other reagent kits such as Nano Orange [15]. In recent years, inductively coupled plasma-mass spectrometry (ICP-MS) has been proposed for the analysis of biomolecules as a powerful tool in proteomics [16–20], and for quantitative analysis of proteins via sulfur determination by high performance liquid chromatography (HPLC) coupled to isotope dilution ICP-MS with a hexapole collision cell [21] using BSA, superoxide dismutase (SOD), and metallothionein-II as model proteins.

The objective of the present research is the development and the study of the analytical performance of a method to analyze BSA and L-cysteine after the determination of sulfur, using high-resolution continuum source molecular absorption spectrometry (HR-CSMAS), and measuring the absorbance of the CS molecule in an air-acetylene flame.

2. Experimental

2.1. Instrumentation

A ContrAA® 300 high-resolution continuum source atomic and molecular absorption spectrometer (Analytik Jena, Jena, Germany) was used to determine the concentration of sulfur.

An Ethos Plus MW Labstation (Milestone, Sorisole, Italy) was used to digest BSA and L-cysteine. The high pressure segmented rotor used in the Ethos Plus MW Labstation contains ten Teflon vessels. The

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maximum operating pressure is 100 bar, and the maximum operating temperature is 300 °C. The vessel's volume is 100 mL.

2.2. Reagents and solutions

The 1 g L^{-1} stock sulfur solution was prepared from the ammonium sodium sulfate salt (Panreac Química, Barcelona, Spain). BSA and L-cysteine were obtained from Sigma-Aldrich (Steinheim, Germany).

96% (w/w) was diluted with water to 3.0% (v/v) (approximately 18 g L^{-1} of S), and this concentrated solution was used for the optimization of flame conditions. 65% (w/w) nitric acid and 33% (w/w) hydrogen peroxide used in the digestions were also purchased from Panreac.

All solutions were prepared using ultrapure water (specific resistance 18 MΩcm) from a Milli-Q purification system (Millipore Corporation, Massachusetts, USA). All glassware and plasticware were kept in nitric acid for 48 h and washed with ultrapure water.

2.3. Digestion of BSA and L-cysteine

Portions of 0.3 g of BSA (or 0.1 g of L-cysteine) were weighed into digestion vessels, and 4.0 mL of 69% (w/w) HNO_3, 2.0 mL of 33% (w/w) H_2O_2, and 2 mL of ultrapure water were added. Afterwards, the vessels were introduced into the microwave oven, where one sample vessel was monitored by pressure control during the whole operation. The temperature program used in the microwave oven lasted 23.5 min at a power of 1000 W and consisted of 4 steps: 1) 2.5 min till 90 °C; 2) 6 min till 140 °C; 3) 5 min till 200 °C; 4) 10 min at 200 °C. When the digestion ended, the content of each reactor corresponding to the L-cysteine digest (or two reactors in case of BSA) was adjusted to its final volume (25 mL) by addition of milli-Q water.

2.4. Sulfur determination using high-resolution continuum source molecular absorption spectrometry

The determination of sulfur was performed using peak volume selected absorbance and the 258.056 nm analytical line [3,7,9,10]. 200 pixels were used for analytical purposes; the five central pixels were used for the determination of sulfur. Pixels number 95–97 and 109–111 were selected for background correction. The 50-mm burner, available in our lab, was used for all the measurements. The operating conditions for the determination of sulfur in the air-acetylene flame are shown in Table 1.

2.5. Analysis of BSA and L-cysteine

The analyses of BSA and L-cysteine were performed taking into account the percentage of S: 1.93 g S/100 g BSA and 26.47 g S/100 g L-cysteine.

Table 1
Operating parameters for the determination of sulfur.

	Value
Analytical line	258.056 nm
Analytical pixels	5 central pixels (CP ± 2)
Correction pixels	95–97, 109–111
Measurement time	25 s
Delay time	15 s
Replicates	3
Height above burner	12 mm
Air flow	7.5 L min^{-1}
Acetylene flow	2 L min^{-1}
Acetylene/air ratio	0.268

3. Results and discussion

3.1. Optimization of flame conditions

Flame conditions were optimized using standards containing 3.0% (v/v) sulfuric acid. The acetylene flow was set at the maximum level (2 L min^{-1}) because a reducing flame is needed for the formation of CS.

The influence of burner height and measurement time was studied. The peak volume selected absorbance (A) (see Fig. S1 (Appendix A)) was measured at different heights above the burner ranging from 10 to 15 mm. The selection of the evaluated interval was decided after the preliminary use of the automatic optimization option allowed by the instrument's software. The maximum signal was observed at 12 mm (see Fig. S1(a) (Appendix A)). Four different measurement times were tested (15, 20, 25 and 30 s). There was no statistically significant difference between the absorbance values obtained at 25 s (1.6479 ± 0.0127) and at 30 s (1.7648 ± 0.6303) at the 95.0% confidence level (*t*-test). Therefore, 25 s was selected as the measurement time (see Fig. S1(b) (Appendix A)).

3.2. Selection of correction pixels

In the preliminary experiments, a calibration with aqueous standard solutions was prepared with concentrations of 0, 100, 200, 300, 400 and 500 mg L^{-1}. The absorbance was measured and the calculations were performed using different correction pixels. Table 2 shows the calibration graphs and the limits of detection and quantification obtained for the determination of sulfur. The limit of detection (LOD) is the minimum concentration of analyte that can be distinguished from the blank. It is defined as LOD = 3 × SDblank/m, where SDblank is the standard deviation of eleven measurements of the blank and m is the slope of the calibration function. Similarly, the limit of quantification is defined as LOQ = 10 × SDblank/m. The lowest LOD (25 mg L^{-1}) was obtained for correction pixels number 95–97 and 109–111 (258.0474–258.0675 nm, 258.0675–258.0704 nm; see Fig. 1), and these were used for correction in the subsequent experiments.

3.3. Addition and calibration graphs. Analytical performance

3.3.1. BSA digested using a MW lab station

The calibration with aqueous standard solutions and the standard addition were prepared with concentrations of 0, 100, 200, 300, 400 and 500 mg L^{-1} of sulfur. To prepare the standard addition, a volume of 15 mL of the MW lab station digests was diluted to 25 mL with water. The equations of the calibration functions were as follows: A = 0.0000127[S] + 0.000296, r = 0.994 (calibration with aqueous standard solutions) and A = 0.0000082[S] + 0.00189, r = 0.994 (standard addition graph). A *t*-test was performed to compare the slopes, and there was a statistically significant difference (95% confidence level), pointing to the existence of the matrix effect.

The limit of detection was calculated using 11 measurements of a digestion blank (SDblank = 4.91 × 10^{-5}); the instrumental LOD for sulfur was 18 mg L^{-1} corresponding to 933 mg L^{-1} of BSA (taking into account the content of sulfur present in BSA: 1.93%). The LOD for S referred to a 0.6 g solid sample was 1.3 mg g^{-1} corresponding to 67 mg g^{-1} of BSA. The analytical recovery in the analysis of BSA was 106 ± 2% (calculated as R(%) = [*BSA*]experimental / [*BSA*]sample × 100), 100), a higher value than that obtained using a conventional MW (81 ± 1%). The use of a torque wrench to secure the vessels used in the Milestone Ethos Plus provides a better seal, which cannot be ensured in the manually closed pumps used in a domestic MW. The use of a MW lab station was therefore the preferred option for digesting the protein. It was also used for the analysis of L-cysteine in later experiments. The repeatability of the analysis was calculated after repeatedly measuring the absorbance of a 280.2 mg S L^{-1} standard (n = 11) that contained