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# Simultaneous detection of selenium by atomic fluorescence and sulfur by molecular emission by flow-injection hydride generation with on-line reduction for the determination of selenate, sulfate and sulfite

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## A B S T R A C T

An inductively coupled plasma atomic fluorescence spectrometry (ICP-AFS) instrument, was modified so that it was capable of monitoring transient chromatographic or flow-injection profiles and that sulfur molecular emission and selenium atomic fluorescence could be monitored simultaneously in an argon–hydrogen diffusion flame on a glass burner. The analytes were introduced as hydrogen selenide and hydrogen sulfide, generated on a flow-injection manifold. Selenate was reduced to hydride-forming selenite by microwave-assisted on-line reaction with hydrochloric acid, and sulfate, or sulfite, was reduced to hydride-forming sulfide by a mixture of hydriodic acid, acetic acid and sodium hypophosphite. The effects of the nature of reducing agent, flow rate, microwave power and coil length were studied. The limit of detection (3 s) for selenium was  $10 \mu\text{g L}^{-1}$ , and for sulfide was  $70 \mu\text{g L}^{-1}$  ( $200\text{-}\mu\text{L}$  injection volume). The calibration was linear for selenium up to  $2 \text{ mg L}^{-1}$  and to  $10 \text{ mg L}^{-1}$  for sulfide. The throughput was  $180 \text{ h}^{-1}$ . The three sulfur species could be differentiated on the basis of reactivity at various microwave powers.

## 1. Introduction

A number of selenium-enriched materials derived from plant sources have anti-cancer properties as well as being suitable sources of the selenium needed to keep healthy [1,2]. There is, therefore, considerable interest in the identification of the organo-selenium compounds in these materials and of the interaction of such compounds with biological systems. Procedures based on high-performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS) detection have been developed [3] but only limited success has been achieved so far with matching of retention times with those of standards because of the limited number of authentic compounds available. The same is true for the coupling of organic mass spectrometry with HPLC separation [4] because of (a) the poorer detection capabilities of such instruments compared to those of ICP-MS and (b) the interferences due to the presence of sulfur analogues in the chromatographic eluent. In addition, reactions in which species that contain both selenium and sulfur are formed are of considerable interest [5–7]. There is, therefore, a need to detect S and Se simultaneously; however, although the detection capability of the quadrupole mass spectrometers currently used with ICP sources, is adequate for the

detection of selenium (once potential isobaric interferences have been taken of), it is insufficient to detect S-containing species reliably, though the formation of oxides in a reaction cell offers some possibilities [8,9]. As an alternative approach to the determination of these elements, it is worth considering the possibilities of optical spectroscopies.

Sulfur can be determined via the absorption or emission characteristics of simple molecular species, such as  $\text{S}_2$ . Selenium may be determined by atomic absorption, emission or fluorescence spectrometry. The relevant species can be produced in cool flames [10] whose temperatures can be controlled by diluting the fuel with an inert gas, such as argon or nitrogen [11]. However, when solutions are nebulized into such flames, the solvent evaporation and sample vaporization are inefficient [12]. One method of avoiding this limitation is conversion of the analyte to a volatile derivative that can then be transported to the flame as a vapor.

In 1975, Thompson described [13] a dispersive atomic fluorescence spectroscopy (AFS) system for the determination of arsenic, selenium antimony and tellurium following hydride generation. The atom source was an argon–hydrogen flame maintained on a Pyrex glass burner. A similar system with a continuous-flow manifold was described by Ebdon et al. [14] for the determination of arsenic and selenium from the appropriate oxidation state precursors (selenium must be in the +4 oxidation state, as  $\text{Se(VI)}$  does not form  $\text{H}_2\text{Se}$  in acid solutions of borohydride). Most of the hydride-forming elements can be detected in the flame in the ultraviolet

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region of the spectrum, below 250 nm, where there is little background. Also, because the analyte has been separated from the matrix, non-dispersive AFS detection is possible. Since Tsujii and Kuga [15] first described the technique in 1974, a number of non-dispersive AFS systems have been developed [16–18]. Corns et al. [19] developed commercial instrumentation based on a study of suitable excitation sources, atom reservoirs, optical configurations, and the optimization of flow-injection hydride generation parameters for the determination of arsenic and selenium. The atom cell was a flame, fueled by hydrogen produced from the decomposition of borohydride (1.5%, m/v) in the flow manifold, sustained on a simple borosilicate glass tube burner.

Sulfur can be detected if introduced into the flame in the form of hydrogen sulfide or as sulfur dioxide (though with a much lower sensitivity). Arowolo and Cresser [12] found that a 5  $\mu\text{g mL}^{-1}$  sample of sulfide produced emission from  $\text{H}_2\text{S}$  that was approximately four times more intense than that from the  $\text{SO}_2$  generated from 100  $\mu\text{g mL}^{-1}$  of sulfite following reaction with hydrochloric acid. The best sensitivity and hence detection limits will only be achieved if the sulfur compounds are reduced to the  $\text{S}^{2-}$  state. As noted above, selenium species must be reduced to the Se(IV) state prior to the hydride generation stage. The reduction of Se(VI) to Se(IV) by hydrochloric acid in a flow-injection manifold has been reported [20–22]. For organo-selenium compounds an oxidation step is required to convert the selenium to selenium (VI) prior to reduction. Several research groups have described [23–26] the on-line microwave-assisted digestion hydride generation of inorganic and organic selenium species.

Arowolo and Cresser [27] described a flow-injection system for the determination of sulfite and sulfur dioxide by cool flame molecular emission after reduction to hydrogen sulfide with borohydride. This overcame the problem of the low emission intensity of sulphur dioxide in the cool flame. Santos and Korn reduced sulfite to sulfide with granulated zinc in 1 M HCl solution [28], with detection, after diffusion through a Teflon membrane, by visible absorption spectrophotometry. Cmelik et al., showed [29] that it was possible to determine sulfite in wine by introduction of the associated sulfur dioxide into an ICP for emission measurements in the vacuum UV.

The reduction of sulfate to hydrogen sulfide is less thermodynamically favored, usually involving the off-line addition of the sample to a strong reducing agent, followed by heating. Even though procedures based on molecular emission cavity analysis (MECA), molecular emission and molecular absorption have been developed for sulfate, the off-line reduction is not easily adapted to flow-injection sample introduction. Rancke-Madsen [30] and Kiba et al. [31] reduced sulfate to hydrogen sulfide with a mixture of tin(II) chloride and phosphoric acid [tin(II) 'strong' phosphoric acid]. Steinbergs et al. [32] used magnesium as the reducing agent for the determination of total sulfur in soil and plant material. Other sulfate reducing agents have involved various hydriodic acid mixes: hydriodic, hypophosphorous, and formic acids [33], hydriodic acid and red phosphorous [34], hydriodic, hypophosphorous and acetic acids [35], as well as hypophosphorous and hydrochloric acids [34].

We have developed, a flow-injection system in which Se(VI) was reduced to Se(IV), and sulfate and sulfite to sulfide. Once converted to the gaseous hydrides, these species were determined in an argon–hydrogen diffusion flame by fluorescence (selenium) and emission (sulfur) spectrometry. A number of reducing agents were evaluated, together with the need for a heat source for which microwave radiation was considered the most suitable. To the best of our knowledge, this is the first report of a spectroscopic determination based on the simultaneous monitoring of the emission from a collisionally excited species and from a photoexcited species. In addition to characterizing the analytical parameters, the possibilities of the system for speciation analysis were evaluated.

**Table 1**  
Summary of optimized system conditions.

Spectrometer	
Modified Baird AFS-2000	Controlled by LabView 3.0.1. Virtual Instrument Software
Se HCL	0.6 A peak current (6 mA average), 1% duty factor, 25 Hz
Se filter	196 nm
S channel	378 nm
Hydrogen diffusion flame	
Burner	4 cm i.d. $\times$ 20 cm long borosilicate glass tube
Hydrogen	200 mL $\text{min}^{-1}$
Argon	600 mL $\text{min}^{-1}$
Optimum viewing height Se	3 mm
Flow-injection manifold	
SAOB carrier	0.1% (m/v) sodium borohydride + 0.1% sodium hydroxide + 0.1% sodium citrate
Reducing agent	2.5 g sodium hypophosphite + 25 mL glacial acetic acid + 100 mL 47% hydriodic acid
Microwave reaction coil	0.8 mm i.d. $\times$ 4 m long
Cooling coil	0.8 mm i.d. $\times$ 2 m long
Stripping coil	0.8 mm i.d. $\times$ 0.6 m long
Carrier flow rate	2.0 mL $\text{min}^{-1}$
Reductant flow rate	4.3 mL $\text{min}^{-1}$
Microwave oven	50% power (reduction of all sulfur species)

## 2. Experimental

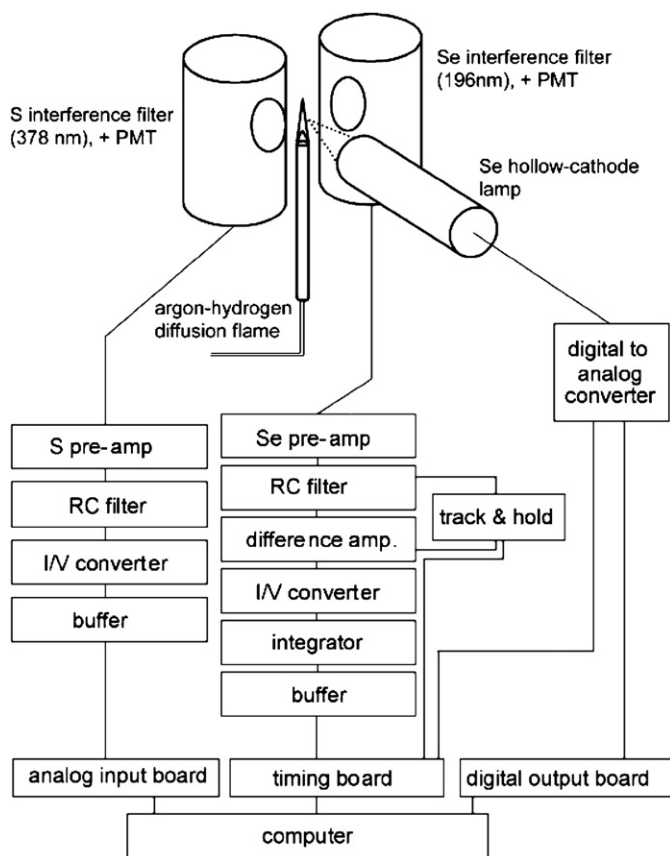
### 2.1. Instrumentation and reagents

#### 2.1.1. Spectrometer

A modified Baird AFS-2000 atomic fluorescence spectrometer (Baird Corporation, Bedford, MA, USA) was used in all selenium and sulfur determinations. The plasma torch was replaced with a burner for a hydrogen diffusion flame. One of the hollow cathode lamp (HCL)/photomultiplier tube (PMT) modules was utilized for the excitation and detection of selenium fluorescence. The PMT detector was fitted with an interference filter at 196 nm. A Baird selenium hollow cathode lamp was mounted at 60° laterally to the selenium PMT. The modified electronics for this system included a pre-amplifier, RC filter, difference amplifier, current to voltage converter, integrator, and buffer [36]. The selenium lamp was pulsed at a 25 Hz with 0.6 A peak current (6 mA average current), and 1% duty factor. System parameters are shown in Table 1.

For the molecular sulfur emission optics, the Baird monochromator unit was initially considered, but proved unsuitable because of low light throughput characteristics. Instead an IP-28 PMT with a Baird thallium interference filter (378 nm) was mounted on one of the Baird PMT module stands. For data collection, the difference amplifier and integrator chips were removed from the one of the 'fluorescence' signal processing boards. Thus the PMT current was converted to a voltage, filtered in a simple RC circuit, and then buffered and digitized using a data acquisition board (National Instruments). The modified spectrometer is shown schematically in Fig. 1. The selenium lamp and 'track and hold' electronics, as well as data collection for both selenium fluorescence and sulfur emission were controlled by LabView version 3.0.1 (National Instruments, Austin, TX, USA), running on a 66 MHz PC (running Windows for Workstations version 3.1.1).

The burner, based on the design proposed by Corns et al. [19], was a borosilicate glass tube (4 mm i.d.  $\times$  20 cm long), mounted in the Teflon plasma torch mount, in the center of the Baird AFS-2000 instrument. The tube was painted matte black to reduce scattering of the light from the selenium HCL. For operation in the mode in which it was fueled by hydrogen from the decomposition of borohydride, the tube was connected directly to the end of the flow-injection manifold. For operation with an external hydrogen source,



**Fig. 1.** Block diagram showing the optics and electronic configuration of the modified Baird AFS-2000 spectrometer for the simultaneous determination of sulfur by molecular emission and selenium by atomic fluorescence.

'T'-piece (2 mm i.d.) was added between the tube and flow-injection manifold to introduce this hydrogen. The top of the spectrometer extraction hood was connected to the extraction duct by a piece of 15 cm i.d. aluminium tubing which also blocked ambient light.

A microwave digestion system, Model MDS-81D (CEM Corporation, Mathews, NC, USA), with an output of  $630 \pm 70$  W in 1% increments, was used as the microwave source. PTFE flow-injection

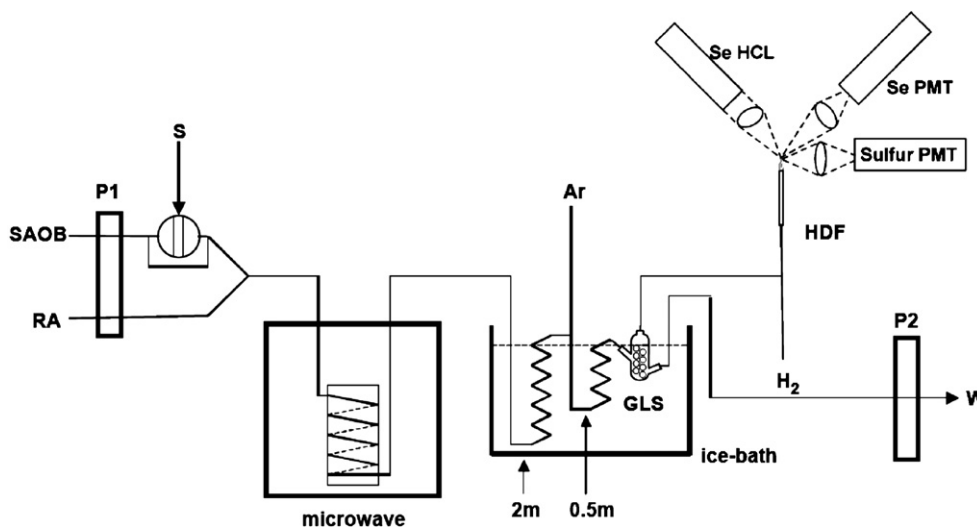
tubing, of various lengths, was wrapped around an inner vessel (a Teflon cylindrical container 4 cm in diameter and 12 cm high) and positioned securely in the center of the cavity.

### 2.1.2. Flow-injection manifold

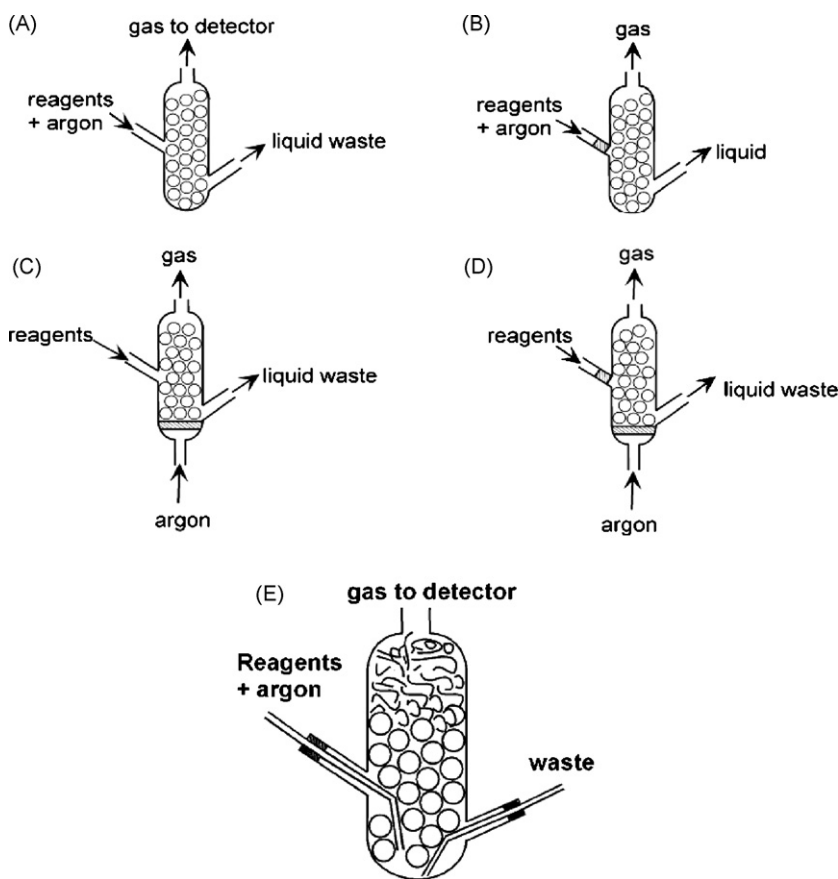
The flow-injection manifold used is shown schematically in Fig. 2. The PTFE tubing prior to the gas-liquid separator (GLS) was 0.8 mm i.d. From the GLS to the hydrogen diffusion flame, the tubing was 1.5 mm i.d. A six-port PTFE Rheodyne rotary valve (Supelco, Bellefonte, PA, USA) was plumbed into the reductant line. An Ismatec SA MS-Reglo Model 7331-10 peristaltic pump (Cole-Parmer, Vernon Hills, IL, USA) propelled the carrier, reagent and sample streams. A second pump drained the GLS. The flow rates were regulated by varying the speed of the pump-head and by changing the i.d. of the Tygon pump tubing when necessary. Following microwave heating, the liquid was cooled by passing the flow-stream through 2 m of 0.8 mm i.d. coiled PTFE tubing submerged in an ice-bath.

A PerkinElmer 'W'-type design gas-liquid separator (GLS), part number B019-3772, was used for most of this work, but several GLS incorporating glass frits, shown schematically in Fig. 3, were also tested. Hoppstock and Lippert [37] fabricated a 'frit-in-base' GLS design specifically for the efficient removal of  $H_2S$  from solution. Each GLS was filled with glass beads of 3–4 mm diameter. For experiments involving microwave heating, the connections to the PerkinElmer GLS were modified so that the inlet and waste-removal tubing extended into the body of the separator (as shown in Fig. 3E). The positioning of the drain-tube close to the base of the GLS has been found to keep the amount of bulk liquid in the separator to a minimum [38]. The glass beads prevented liquid transfer to the atomizer and by submerging the GLS in the ice-bath, carry-over of water vapor following the heating step was further minimized. It is possible that other designs of GLS would be suitable; however, previous studies [39] have shown that the best design may be dependent on the analyte and operating conditions.

For initial studies involving characterization of the Se/S detectors, hydrochloric acid was used and a PermaPure Nafion<sup>®</sup> dryer, model MD 110-12F (30 cm long, 2 mm i.d.) (Toms River, NJ, USA), was connected between the top of the gas-liquid separator and the base of the borosilicate glass atomizer to remove water vapor. As the Nafion distorted under the action of the various acid vapors produced from the strong reducing agents used in the microwave-



**Fig. 2.** Optimized flow-injection manifold for the microwave-assisted reduction of sulfur and selenium compounds: SAOB is the standard anti-oxidant buffer carrier stream ( $2.0 \text{ mL min}^{-1}$ ), RA is the hydriodic/acid acetic/hypophosphorous acids reagent stream ( $4.3 \text{ mL min}^{-1}$ ), W is the waste stream. Flow rates for pump 1 (P1) are shown in  $\text{mL min}^{-1}$ . The sample solution, S, is introduced via a 1000- $\mu\text{L}$  injection loop, Ar represents the introduction of argon ( $600 \text{ mL min}^{-1}$ ),  $H_2$  is the introduction of hydrogen ( $200 \text{ mL min}^{-1}$ ). GLS is the gas-liquid separator and HDF is the hydrogen diffusion flame.



**Fig. 3.** Gas-liquid separator designs. A-D represent designs incorporating glass frits. Design E was chosen as the best. The body of the separators are filled with beads topped off, in the case of E, with mm-size crushed glass spirals. The designs are not shown to scale.

assisted reduction experiments, the dryer was removed for these experiments.

The argon flow was controlled by a Type 1179A Mass-Flo<sup>®</sup> controller (MKS, Andover, MA, USA), already calibrated for argon by the manufacturer. For the experiments in which an external supply of hydrogen fueled the flame, the argon gas flow rate was controlled by the needle valve of a rotameter, and the hydrogen flow was controlled by the mass-flow controller (MFC). The MFC was re-set by a factor recommended by the manufacturer to account for the difference in densities between hydrogen and argon. The error in this factor is, according to the manufacturers, less than 10%.

### 2.1.3. Data handling

Data for the Se fluorescence signal and S emission signal were collected at 25 Hz, saved as an ASCII file, and imported into PeakFit version 4 (SPSS Science Software Ltd., Edgbaston, U.K.) so that peak areas could be calculated. Peak area measurement was chosen in preference to peak height measurement because it was more precise. A simplex optimization using MultiSimplex TM software (MultiSimplex KB, Karlskrona, Sweden) found the optimal optical conditions for both the sulfur PMT, and the selenium PMT and HCL. For these experiments, data was collected for 20 s in a continuous-flow mode (rather than flow-injection mode). An EXCEL spreadsheet (Microsoft) was used to find the signal/standard deviation of the blank for each set of simplex conditions before the result was entered into the MultiSimplex software.

## 2.2. Reagents

All solutions were prepared with distilled, deionized water produced by an E-Pure System (Barnstead). Hydrochloric acid solution

was prepared by diluting the appropriate amount of reagent ACS grade concentrated hydrochloric acid (36.5%, m/m, Fisher Scientific) with distilled, deionized water. A standard anti-oxidant buffer solution (SAOB) based on that used in sulfide determinations by Ebdon et al. [40], was prepared by dissolving sodium borohydride (98% minimum assay Alfa Aesar, Ward Hill, MA, USA) and sodium citrate (Fisher Scientific) in a solution of sodium hydroxide (ACS reagent-grade, Mallinckrodt, Paris, KY, USA) to give a solution containing 1.5% (m/v) NaBH<sub>4</sub>, 0.1% (m/v) NaOH, and 0.1% (m/v) citrate. Lower concentrations of borohydride were used when microwave heating was applied. For a borohydride concentration of 1.5%, segmentation was excessive.

Standard selenium solutions (1000 mg L<sup>-1</sup>) were prepared by dissolving the 0.333 g of sodium selenite (Fisher Scientific), and 0.239 g of sodium selenate (Fisher Scientific) in separate 100 mL of distilled, deionized water. Solutions of sulfate, sulfite and sulfide (5000 mg L<sup>-1</sup>) were prepared by dissolving 2.22 g of sodium sulfate (Fisher Scientific), 1.97 g of sodium sulfite (Fisher Scientific), and 3.84 g of sodium sulfide (Na<sub>2</sub>S·9H<sub>2</sub>O) (VWR Scientific), in 100 mL distilled, deionized water. Standards of lower concentrations were prepared by dilution with standard anti-oxidizing buffer solution, even though such solutions are not very stable and may need to be prepared more frequently than solutions of higher oxidation states.

### 2.2.1. Preparation of tin(II) 'strong' phosphoric acid

Two methods of preparing the tin(II) 'strong' phosphoric acid were considered. The first preparation proposed by Bogdanski et al. [41] involved the addition of 240 g of tin metal to 1200 mL of orthophosphoric acid, followed by distillation. Because the purity of the tin metal available was unknown, a second method proposed by Kiba et al. [31] was adopted. In this procedure, 50 g of



$\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$  was added to 200 g of orthophosphoric acid. Since the density of the orthophosphoric acid was  $1.75 \text{ g L}^{-1}$ , the volume was 114 mL. The tin(II) chloride was ACS certified grade (Fisher Scientific), and it was considered that this procedure would yield a reagent with lower concentrations of impurities. Following addition of the tin(II) chloride to the orthophosphoric, the solution was boiled under reflux for 2 h. The resulting reagent was too viscous for use in the flow-injection manifold. On dilution with water (1 + 3), the resulting solution could be pumped through the manifold.

### 2.2.2. Preparation of hydriodic/hypophosphorous/acetic acid reducing agent

The reagent, suggested by Gustafsson [35], was prepared by dissolving sodium hypophosphite, 2.5 g (Fisher Scientific) in 25 mL of ACS reagent-grade glacial acetic acid (Mallinckrodt) in a round-bottomed flask fitted with a ground-in condenser and a gas delivery tube followed by the addition of hydriodic acid (100 mL of 57%, v/v Alfa Aesar). The hydriodic acid was always taken from a freshly opened flask. The solution was boiled under reflux for at least 2 h, while a stream of argon gas was bubbled through the solution (about  $50 \text{ mL min}^{-1}$ ) to remove traces of sulfur. During this time, the color of the solution changed from brown to pale yellow, as the iodine was reduced to iodide. This solution was cooled in argon stream, the flask was closed with a glass stopper and stored in the dark.

## 2.3. Method development

### 2.3.1. Manifold optimization

The parameters that were considered to influence the analytical performance were the usual flow-injection parameters of sample volume, flow rates and reagent concentrations, total flow rate, and nature of the gas liquid separator. In addition the observation height, the source of hydrogen (borohydride decomposition or externally supplied) and the degree of pulsation produced by the pumps were also considered to affect the signal to noise ratios. The optical parameters and the flow-injection parameters were considered to be linked by the hydrogen flow rate which was expected to influence the heights in the flame at which maximum emission and maximum fluorescence were obtained. The quantitative figure of merit was peak area signal sensitivity, while signal pulsations were evaluated qualitatively. Conditions that produced excessive pulsations were considered sub-optimal.

Following optimization of the manifold for the determination of selenium as selenite, and sulfur as sulfide, the reduction of higher oxidation states by the various reducing agents and the role of microwave-assisted reduction were investigated. The parameters studied included the concentration of the reagent, length of time in the microwave field which in turn depended on tube length, flow rate and power setting.

Initially the flow-injection manifold (shown in Fig. 2), was used with higher concentrations of hydrochloric acid in the acid line. Concentrations starting at 6 M, and then up to 12 M were tested for the ability to reduce Se(VI) to Se(IV), and sulfate and sulfite to sulfide. These experiments were also repeated with a microwave oven in place between the confluence point of the acid and SAOB streams, and the GLS. A 10 m knotted reaction coil (PerkinElmer) was used as the reaction coil placed inside the microwave cavity.

For the hydriodic acid based reagent, coil lengths of 2, 4 and 10 m, and flow rates of 2.2, 4.3, 6.0, 8.9, and  $11.4 \text{ mL min}^{-1}$  were used. Not all of the possible combinations of variables were tested due to the high cost of this reagent. Power settings between 0% and 70% were investigated. In several experiments this range included values at which the solution boiled.

### 2.3.2. Method performance

The spectroscopic interferences were evaluated. These included the influence of the sulfur emission on the selenium channel, and vice versa, and of HPO emission on the sulfur channel. Calibrations for selenite and sulfide were obtained and estimates made for the detection limits of these two species. The possibility of devising a procedure for the determination of the three sulfur species in admixture based on reaction with the three-acid mixture reductant under different microwave conditions was evaluated. The same evaluation was made for the two selenium species with hydrochloric acid as the reductant.

## 3. Results and discussion

### 3.1. Method development

Better performance was obtained from the PerkinElmer GLS shown in Fig. 3E than from any of the designs with frits in the inlet channel or the base. The position of the inlet and outlet tubes was found to be a significant feature and the positions shown in Fig. 3E gave the best performance. The signal increased approximately linearly with sample volume. The response for various designs as a function of stripping gas flow rate, in the absence of any additional hydrogen, is shown, for sulfur, in Fig. 4. With the frit-in arm GLS design (Fig. 3B) and argon stripping gas flow rates of  $100 \text{ mL min}^{-1}$  or higher, the flame was extinguished. The response for selenium (not shown) was very similar.

Although it was possible to obtain signals for both elements from the flame fueled by the internal generation of hydrogen, the operating parameters could be varied only over a rather limited range of values before the flame became unstable, lifted off or dropped below the burner rim. The maximum argon flow rate was  $200 \text{ mL min}^{-1}$ . There were significant fluctuations in signal, which were decreased by the incorporation of pulse dampers, but were not influenced by the geometry of the confluence point.

Better performance was obtained from the system in which an external hydrogen supply was used. At a hydrogen gas flow of  $200 \text{ mL min}^{-1}$ , argon flows could be increased to  $600 \text{ mL min}^{-1}$ , which increased the height of the flame from 1.5 cm to 4 cm. This

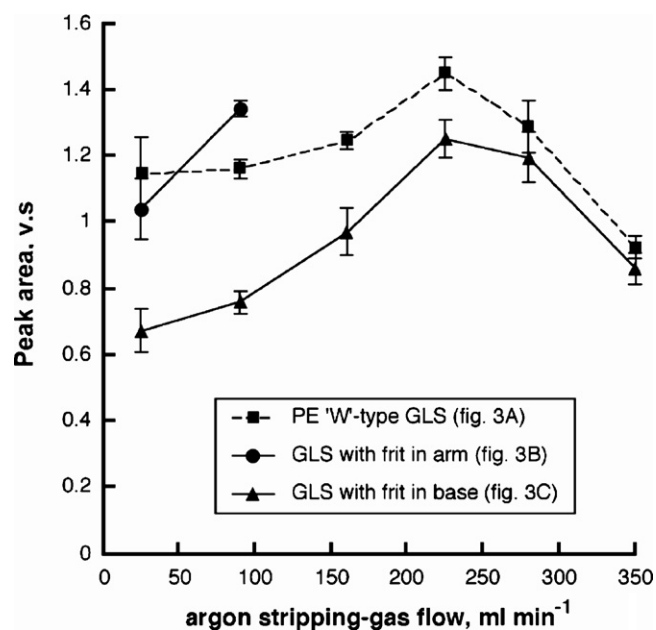


Fig. 4. Plot of variation in signal as a function of argon flow rate for a  $200 \mu\text{L}$  injection volume of  $2 \text{ mg L}^{-1}$  sodium sulfide. Error bars represent standard deviations ( $n = 4$ ).

allowed spatial separation of the sulfur and selenium signals so that the spectral overlap observed for the smaller flame (the signal of  $100 \mu\text{g L}^{-1}$  sulfur was increased by 20% in the presence of  $1 \text{ mg L}^{-1}$  selenium) was eliminated. The signal to noise ratio increased approximately linearly with argon gas flow for a variety of dimensions of stripping coils. The best value was obtained for a 60-cm coil of internal diameter 0.8 mm.

The detection limits, for a 200- $\mu\text{L}$  sample volume, were  $10 \mu\text{g L}^{-1}$  for selenite and  $70 \mu\text{g L}^{-1}$  for sulfide for the internally fueled system and  $2 \mu\text{g L}^{-1}$ , and  $15 \mu\text{g L}^{-1}$  for the species, respectively for the externally fueled system. The upper limit of the calibration was  $2 \text{ mg L}^{-1}$  for selenium and  $100 \text{ mg L}^{-1}$  for sulfur, though this decreased to about  $10 \text{ mg L}^{-1}$  for the externally fueled system. The throughput, based on peak width, was  $180 \text{ h}^{-1}$ .

### 3.2. Microwave-assisted reduction of sulfur and selenium species

Hydrochloric acid, 6 M, reduced Se(VI) to Se(IV) at microwave power settings of 20–30%. Concentrated hydrochloric acid (12 M) fully reduced Se(VI) to Se(IV) without the use of microwave radiation, whereas the reduction of sulfite to sulfide was only partial; 30% power was needed for complete reduction. No signal was obtained for sulfate at any microwave power setting for hydrochloric acid.

The main disadvantage of the diluted tin(II) orthophosphoric acid was that the reducing power had been greatly diminished by dilution [30]. The other disadvantage was that the production of phosphoric acid vapor had to be controlled. If the GLS was not properly cooled, phosphoric acid vapor would carry over to the flame, and the green HPO emission was observed causing a positive bias in the sulfate signal due to spectral overlap. The three-acid mixture had a much lower viscosity than the concentrated tin-phosphoric acid reagent, and could be pumped through the flow-injection manifold.

The experiments with various coil lengths and flow rates showed that for a 2-m coil, there were problems at both high and low flow rates. At high flow rates the conversion of sulfate to sulfide was poor; at low flow rates, the reproducibility was poor ( $\text{RSD} > 10\%$ ). For the 10-m coil, although reduction of sulfate to sulfide was possible, unless very high flow rates were used, the segmentation produced when the microwave power was sufficiently high to cause sulfate reduction gave rise to erratic flow to the gas-liquid separator producing multiple peaks and poor precision. Typical peak shapes for a 1000- $\mu\text{L}$  injection of  $10 \text{ mg L}^{-1}$  sulfate at various microwave power settings are shown in Fig. 5. This rather large injection volume was decreased to a more typical volume of 200  $\mu\text{L}$ , when analytical figures of merit were determined. The peaks at 40% and 50% microwave power are broad, which suggests that it may be possible to decrease the injection volume considerably. Peak shapes were erratic when the microwave power was increased above 50%; the solution boiled and the flow into the gas-liquid separator was no longer uniform. When the flow rates were decreased from  $6 \text{ mL min}^{-1}$  to 2–3  $\text{mL min}^{-1}$ , the peak areas increased; however, peak shape and reproducibility were poor.

The signal from a mixture of  $10 \text{ mg L}^{-1}$  sulfide and  $10 \text{ mg L}^{-1}$  sodium sulfate under microwave-on and microwave-off conditions showed that with the microwave-off, only sulfide was detected, whereas with the microwave-on both sulfide and sulfate were detected. Hence it would be possible to determine these two species based on a simple microwave-on/microwave-off strategy.

Of the coil lengths investigated, the best results were obtained with the 4-m coil. The sulfur signal resulting from the reduction of sulfate in the 4-m coil at various flow rates as a function of microwave power all showed two maxima. For each flow rate, the decrease after the first maximum coincided with the onset of boiling. Below the boiling point, the flow of liquid was uniform; whereas when boiling occurred, the flow rate fluctuated and mis-shaped

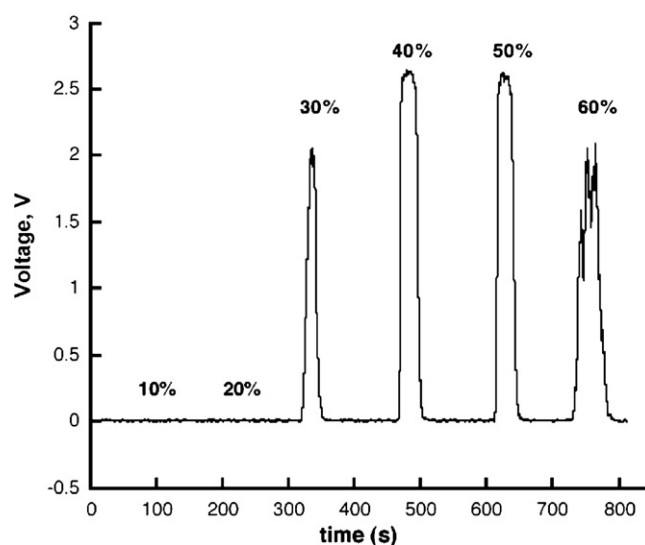


Fig. 5. Responses for five injections of 1000  $\mu\text{L}$  of  $10 \text{ mg L}^{-1}$  sulfate at various microwave power settings (% of 630 W). The reductant was the hydriodic/acetic/hypophosphorous acids reagent. The peaks are shown displaced on the time axis for clarity. As can be seen from the figure, the blank signal was only a few mV.

peaks were formed. Above the boiling point, the sample and reducing agent left the microwave cavity as vapor and the flow was again uniform. Conversion of liquid to gas within the manifold (as the solution boiled), followed by condensation (as the fluid passed through the ice-bath) produced uniform flow to the GLS. At the lower flow rate of  $2.2 \text{ mL min}^{-1}$ , a decreased signal was obtained with poorer signal to noise ratio. However, for the relatively low flow rate of  $4.3 \text{ mL min}^{-1}$ , and 20% power, the conversion of sulfate to sulfide was approximately 90%.

The possibility of determining all three sulfur species in a mixture was investigated for the 10-m coil. The results are shown in Fig. 6. At zero microwave power, the signal was almost entirely due to sulfide though a small signal (approximately 5% of the sulfide signal) from sulfite was observed. As the microwave power was

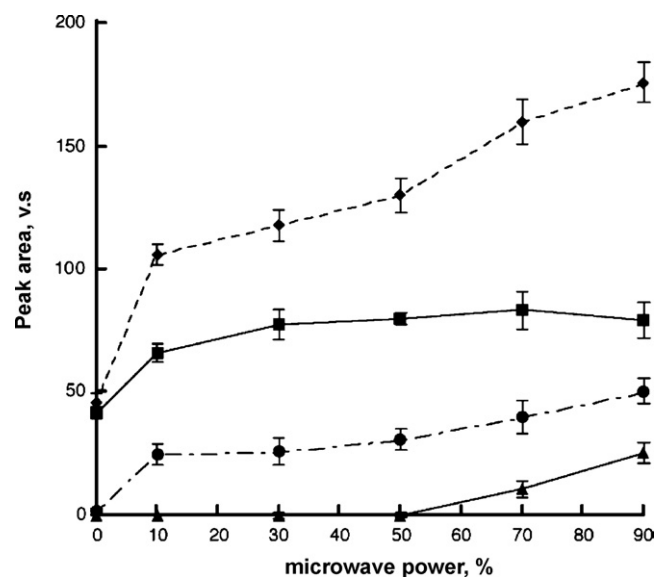


Fig. 6. Plots of peak areas for a 1000  $\mu\text{L}$  injection of  $10 \text{ mg L}^{-1}$  of each sulfur compound (■ sulfide, ● sulfite, ▲ sulfate) and a mixture (◆) of  $10 \text{ mg L}^{-1}$  of each compound as functions of microwave power. The reaction coil was 10 m and the SAOB and three-acid mix reducing agent flow rates were both,  $6 \text{ mL min}^{-1}$ . Error bars are standard deviations ( $n = 3$ ).

increased, the signal from sulfite increased to about 40% of that due to sulfide, and showed a constant response between 10 and 40%, and an increasing response from 40% to 90%. No signal was observed from sulfate until 50% power was applied. Thus a range of power (10–40%) can be identified within which it would be possible to determine sulfide and sulfite, for powers approaching 90% it would be possible to detect all three species, though quantification would require careful calibration to account for the different sensitivity for each species. Such an approach has been demonstrated previously [36].

For all coil lengths, an increase in response for sulfide was observed as the microwave power increased to 10% due most likely to the increases in separation efficiency of the gas–liquid separator as the solution temperature increases.

Conditions were not found under which the conversions of sulfite and sulfate to sulfide were 100%; although 80–90% conversions were obtained for various combinations of flow rate, coil length and microwave power. These values are in line with those encountered in other chemical vapor generation procedures, such as hydride generation, and are not considered to be a major limitation of the method.

#### 4. Conclusions

For a hydride generation atomic fluorescence procedure with a hydrogen diffusion flame atomizer, better performance can be obtained if an external source of hydrogen is used rather than relying solely on the hydrogen produced by the decomposition of the excess borohydride. The incorporation of glass frits into the gas–liquid separator does not lead to improved performance, whereas the careful placing of the inlet and outlet tubings within the body of the separator does.

It is possible to determine sulfide and selenate/selenite by hydride generation with borohydride acidified with hydrochloric acid in flow-injection system with no additional input of energy in which the species produced in a hydrogen diffusion flame are detected by emission (from molecular  $S_2$  species) and fluorescence (from atomic Se). With the addition of microwave energy, the suite of analytes can be extended to include sulfite.

It is not possible to determine sulfate unless a stronger reducing agent is used. A mixture of hydriodic, hypophosphorous and acetic acids will reduce sulfate to sulfide in a flow-injection manifold provided microwave-assisted heating is supplied. There is a microwave power threshold after which sulfate is reduced and prior to which only sulfide and sulfite are detected. Thus it is possible to devise a procedure in which all three sulfur species could be detected, although the calibration of such a procedure would be somewhat complicated. The detection limits achievable ( $2\text{--}20\ \mu\text{g L}^{-1}$ ), while inferior to those of vapor generation with measurement by AAS or AFS, are adequate to support studies involving selenium-enriched plants and yeasts, and the procedure is suitable as a post-column reaction scheme, when likely interferences from some metal ions would be avoided.

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