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A multisyringe flow injection method for the automated determination of sulfide in waters using a miniaturised optical fiber spectrophotometer

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Abstract

In this paper, a fully software-controlled multisyringe flow injection (MSFIA) spectrophotometric system is proposed for the determination of sulfide in environmental and waste waters. The implementation of ancillary solenoid valves into the flow network allows a multitude of injection modalities to be explored, the selected modality being directly dependent on the aim of the assays. The multicommuted sandwich-type approach is introduced in this work as an efficient means to warrant high sensitivity for the particular assay with excellent repeatabilities and a considerable reagent saving. Moreover, a high injection frequency may be easily attained by carrying out a multiple injection modality during a single forward displacement of the piston driver bar. The interfacing of the robust and versatile multisyringe piston pump with an optical fiber plug-in spectrophotometer furnished with a light emitting diode enables the miniaturization of the flow analyzer, which is thus readily adaptable to in-situ and real-time monitoring schemes. The flow method is based on the coupling Fischer's reaction of sulfide with *N*,*N*-dimethyl-*p*-phenylenediamine in the presence of Fe(III) as oxidizing reagent in a 0.7 M HCl medium. Careful selection of the physical and chemical variables enabled coefficients of variations better than 1.5% ($n = 10$) at the 1 mg l^{-1} level for both injection modalities. Dynamic working ranges of 0.2–2.0 and 0.5–5 mg l^{-1} sulfide for sandwich and multiple injection techniques, and detection limits of 0.09 and 0.15 mg l^{-1}, respectively, were obtained. Furthermore, the sandwich modality featured an average slope of 0.43 ± 0.02 l mg^{-1} calculated from 10 day-to-day calibration plots. This result reveals better sensitivity than other flowing stream methods described in the literature. The multiple injection technique allowed an improvement of the injection throughput up to 80 h^{-1}, although a decrease of sensitivity was concomitantly observed (average slope of 0.17 ± 0.01 l mg^{-1}).

The multisyringe flow method was successfully applied to the determination of sulfide in different spiked water matrices (namely, mineral, tap, freshwater, seawater and wastewater) with recoveries ranging from 96 to 104%. Good agreement was also found in water samples between the MSFIA results and those of the batch APHA-standard method.

Keywords: Multisyringe flow injection analysis (MSFIA); Sulfide; Water samples; Miniaturized optical fiber-spectrophotometer

1. Introduction

Recently, a new technique so-named multisyringe flow injection analysis (MSFIA) has been designed [1,2] and presented as a powerful tool for the automatic microfluidic handling of solutions. Its main goal is to obtain full benefit of the versatility and ruggedness of sequential injection schemes using syringe pumps as liquid drivers. Thus, the continuous re-calibration procedures required in flow injection systems as a consequence of the ageing of the flexible Tygon tubing are circumvented, yet maintaining the more efficient radial mixing of zones of flow injection methods. Moreover, the coupling of four soldered piston pumps with solenoid valves enables a multitude of different injection modalities to be readily exploited, such as multicommutation protocols involving binary sampling or tandem-flow, splitting techniques, sandwich schemes and hydrodynamic injections [3–8], as recently reviewed [9]. The implementation of three-way commutation valves at the head of each syringe allows the injection of precise, well defined volumes of sample and reagents according to the time schedule for the analytical measurements. Reagents are dispensed back to the respective reservoirs when not needed without disturbing the reaction development. Hence, more than 10-fold

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reduction of waste generation with regard to common flow injection procedures is typically attained. Besides, the variety of available syringes with capacities comprised between 0.5 and 25 ml enables the performance of different flows and volume injections through the individual network lines. It should be also stressed that the speed of the steeper motor has been adjusted to a wide range, so that reactions with different kinetic demands can be easily accommodated without manifold re-configuration [9].

Reduced sulfur compounds, such as hydrogen sulfide, are found in natural and wastewaters. From the environmental point of view, hydrogen sulfide is one of the most important parameters to monitor in water matrices due to its high toxicity for aquatic organisms. Besides, hydrogen sulfide controls the bioavailability of heavy metals in anoxic environments due to the low solubility of sulfide salts [10]. In wastewater and anoxic aquatic environments, hydrogen sulfide is originated from decomposition of organic matter and reduction of sulfate mediated by bacteria [11], thus being a relevant species for pollution assessment.

One of the most successful and frequently used method for sulfide determination as a consequence of its inherent specificity relies upon the oxidative coupling of sulfide ions with *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) in the presence of Fe(III)-Fischer's reaction—to form the methylene blue (MB) dye. The mechanism of the reaction is described by several authors [11–13].

The aim of this work is to develop a MSFIA procedure, for the spectrophotometric determination of sulfide in natural and wastewaters, taking benefit from the selectivity of the above mentioned reaction in which sulfide ions are converted into methylene blue. A novel sandwich-type multicommuted injection is designed and successfully applied to improve the sensitivity and reliability of the methodology, while a multiple injection modality is developed in order to achieve a high sampling frequency. Various configurations are also evaluated attending both reactions involved in the generation of the coloured species (namely amine oxidation and nucleophilic coupling of sulfide) capitalized on the utmost versatility of the flow technique. The interfacing of the flow set-up with a straightforward and miniaturized fiber optical-based diode-array spectrophotometer furnished with a light emitting diode (LED) warrants the applicability of the automatic methodology to in-field analysis and real-time monitoring of the target analyte, extensible to other parameters of environmental interest by suitable selection of the LEDs.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade and solutions were prepared with distilled water, which was boiled prior to preparation of the entire set of standards and reagents to make it free from dissolved oxygen.

The chromogenic reagent was prepared by dissolving 2.16 g of DMPD, (Sigma) in approximately 50 ml of water, to which 30 ml of 5 M HCl are added before diluting to 100 ml with water. A 50 mM DMPD working solution was prepared by suitable dilution of the stock solution with 1.5 M HCl.

The oxidizing reagent consists of 9.64 g of $NH_4Fe(SO_4)_2\cdot 12H_2O$ (Scharlau) dissolved in a mixture of 50 ml of water and 36 ml of 5 M HCl and made up to 100 ml with water. A 60 mM Fe(III) solution was prepared by proper dilution of the stock with 1.8 M HCl.

A 0.7 M HCl solution prepared from concentrated hydrochloric acid (Scharlau) was used as carrier stream.

The sulfide stock standard solution (ca. $1000\,mg\,l^{-1}$) was prepared daily by dissolving 0.75 g of $Na_2S.9H_2O$ 95% (Panreac) in 50 ml of a 0.05 M NaOH solution and diluting to 100 ml with water. The final solution was daily-standardized iodimetrically [11] and working standard solutions ($0.2–2\,mg\,l^{-1}$) were prepared by suitable dilution of the stock with 0.025 M NaOH.

2.2. Flow system, optical detector and software

The multisyringe flow injection manifold devised for the spectrophotometric determination of sulfide is shown in Fig. 1. A multisyringe piston pump (Crison, Barcelona, Spain) equipped with four syringes S1-S4 (Hamilton, Switzerland) of 2.5, 1, 1 and 5 ml, respectively, was used as a liquid driver. Each syringe has a three-way solenoid valve (N-Research, Caldwell, NJ, USA) at the head (E1–E4), which facilitate the application of multicommutation schemes. The automatic control of the flow device via PC was made through a RS232C interface. The multisyringe module also comprises an additional discrete solenoid valve (model MTV-3-N 1/4 UKG, Takasago, Japan) (E5), which directs the aspirated zones from the autosampler (Crison) to the specially designed five-way PMMA-connector (see Fig. 1 for further details). Loops and connections were made from PTFE tubes of 0.8 mm i.d. The length of the holding coil (HC) and knitted reactor (KR) were 3 m and 122 cm, respectively. The latter coil was constructed by interlacing the PTFE tubing in knots of ca. 5 mm diameter.

The miniaturized optical detector involves a diode-array PC-plug-in spectrophotometer with a PC 2000 A/D card (Ocean Optics, Dunedin, FL) inserted in a PC slot and connected to a glass flow-through cell (10 mm optical path and 36 μl inner volume) via a single optical fiber with a core diameter of 400 μm. The analytical wavelength selected for monitoring the MB dye is 666 nm. A light emitting diode (red diode, 15 mA maximum intensity, spectral band of 600–700 nm, Sciware, Palma de Mallorca, Spain) with a power supply of adjustable intensity (Sciware) was selected as light source.

Instrumental control and acquisition of spectrophotometric data were performed using the software package Auto-

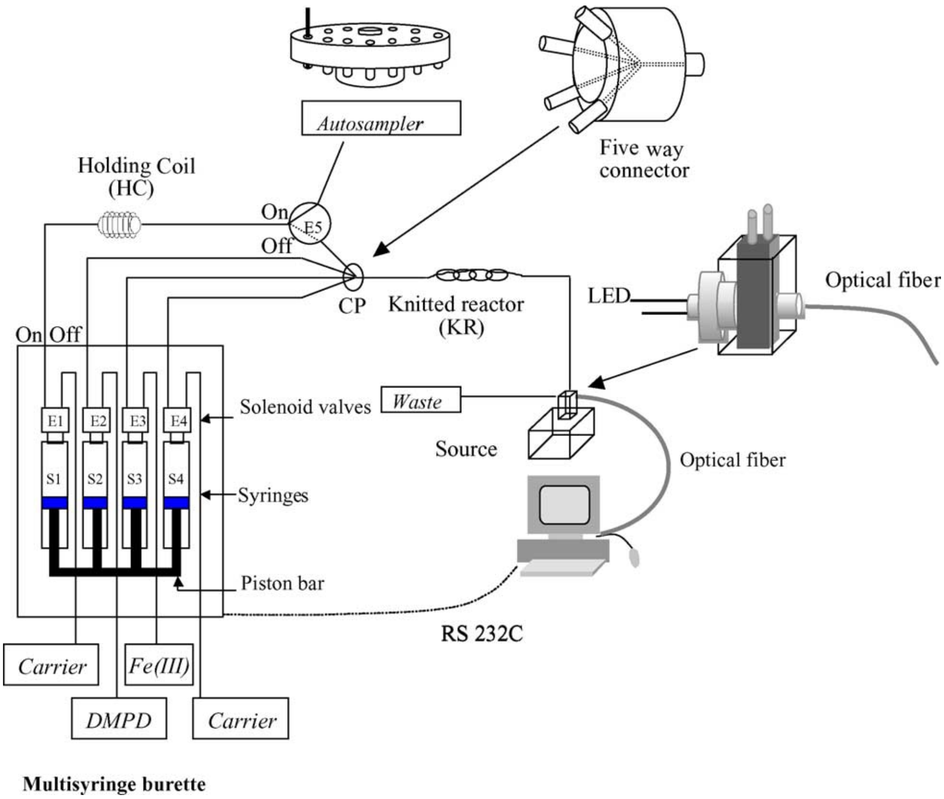

Fig. 1. Schematic representation of the multisyringe flow injection manifold designed for the determination of sulfide in environmental and wastewaters. DMPD: *N*,*N*-dimethyl-*p*-phenylenediamine monohydrochloride; LED: light emitting diode; CP: confluence point.

Analysis 5.0[1] [14] (Sciware, Spain). The distinguished feature of the developed software based on dynamic link libraries (DLLs) at 32 bits is the viability to use a single and versatile application without further modification for whatever instrumentation and detection system needed. It involves a basic protocol, which allows the implementation of specific and individual DLLs attending the configuration of the assembled flow analyzer. In our particular case, the principal protocol was loaded with the DLLs designed for the automatic control of the multisyringe device and diode-array photometer.

2.3. *Multicommutation protocol for sulfide monitoring*

The noteworthy feature of the proposed configuration is the feasibility to evaluate different injection modalities using minute volumes of sample and reagents, which may be dispensed either simultaneously or sequentially into the flow manifold at a variety of flow rates.

2.3.1. *Sandwich technique*

A novel multicommutation protocol involving the simultaneous injection of sample and reagents sandwiched by two additional sample slugs was adopted (see Table 1).

The automated protocol for the spectrophotometric MSFIA-multicommuted determination of sulfide using a sandwich-based injection protocol may be summarized as follows:

1. Initially, the syringes are filled with solutions from the respective reservoirs at 5.0 ml min^{-1} (for syringe 1) with heads 1-On 2-Off 3-Off 4-Off 5-On. The activation of valve five enables the synchronous loading of the holding coil with 450 μl of sample.
2. Thereafter, syringe 1 is set to dispense 0.45 ml at 6.0 ml min^{-1}, while executing the sandwich-type multicommutation command detailed in Table 1. In this step, sample and reagent zones are stacked in the reaction coil during a single forward displacement of the piston driver bar.
3. The diode-array spectrophotometer starts data acquisition at 1 Hz.
4. In order to record the transient analytical signals, syringe 1 with heads 1-Off 2-Off 3-Off 4-On 5-Off is programmed to propel a metered carrier volume (0.65 ml) into the reaction coil at 2.0 ml min^{-1}. Thus, the different plugs are interdispersed downstream, taking place Fischer's method reactions. The excess of carrier delivered during this stage is aimed to rinse the reactor, rendering the system ready for a new analytical cycle. The carrier is dispensed by two syringes (namely, S1 in the

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[1] May be requested at: http://www.sciware-sl.com. http://www.sciwaresl@yahoo.es.

Table 1
Multicommuted sandwich-based injection protocol designed for the spectrophotometric determination of sulfide by MSFIA

Multicommutation step	Solenoid valve schedule					Sample (μl)	Reagents (μl)		Carrier (μl)	Total flow rate (ml min^{-1})
	E1	E2	E3	E4	E5		R_1	R_2		
Front zone	On	Off	Off	Off	Off	125	–	–	–	6.0
Simultaneous injection of sample and reagents	On	On	On	Off	Off	200	80	80	–	6.0
Tailing zone	On	Off	Off	Off	Off	125	–	–	–	6.0
Carrier injection	On	Off	Off	Off	Off	–	–	–	1200	4.0

R_1: DMPD solution; R_2: Fe(III) solution.

multicommutation protocol, and S4 in the final step) in order to make use of a single forward displacement of the piston driver bar. On the other hand, the utilization of a syringe with a great volume would cause a loss of precision.

2.3.2. Multiple injection modality

A multicommutation protocol involving the simultaneous injection of sample and reagents was adopted (see Table 2). The automated protocol for the spectrophotometric MSFIA-multicommuted determination of sulfide by a multiple injection modality may be summarized as follows:

1. Initially, the syringes are filled with solutions from the respective reservoirs at 5.0 ml min^{-1} (for syringe 1) with heads 1-On 2-Off 3-Off 4-Off 5-On. The activation of valve five enables the synchronous loading of the holding coil with 700 μl of sample. The holding coil is filled with an excess of volume, in order to avoid sample dispersion and attain the same height in all peaks.
2. The diode-array spectrophotometer starts data acquisition at 1 Hz.
3. Thereafter, syringe 1 is set to dispense 0.20 ml at 6.0 ml min^{-1}, while executing the multicommutation command detailed in Table 2. In this step, sample and reagent zones are stacked in the reaction coil during a single forward displacement of the piston driver bar. In order to detect the transient analytical signals a metered carrier volume is additionally propelled into the reaction coil. Hence, the different plugs are interdispersed downstream, taking place Fischer's reactions. The excess of carrier delivered during this stage is aimed to rinse the reactor, rendering the system ready for the consecutive injection.
4. Step 3 is repeated three times during a single forward displacement of the piston driver bar, thus obtaining three injections (multi-peak strategy) in the same analytical protocol. The three peaks are averaged to make the calibration and quantification.

3. Results and discussion

Different flow arrangements have been assessed, and a simple configuration relying on merging sample and reagent plugs at a single confluence point has been adopted.

3.1. Manifold configuration

Preliminary experiments were conducted to select the proper configuration for the MSFIA assembly. Two flow arrangements were explored and compared. Firstly, a consecutive merging zones scheme involving the in-line mixing of both reagents prior to merge with the sample segments was assembled. Although this is the common approach exploited in flow injection determinations [12,15–17], no significant differences in terms of sensitivity and coefficients of variation were found in comparison with a unique configuration based on merging the sample and the different reaction ingredients at a single confluence point (see Fig. 1). The latter manifold network was selected for further investigations attending its inherent simplicity and notable analytical features achieved.

3.2. Investigation of chemical variables

The optimum conditions for the in-line color development of Methylene Blue (MB) were assessed. The

Table 2
Multicommuted multiple injection protocol designed for the spectrophotometric determination of sulfide by MSFIA

Multicommutation step	Solenoid valve schedule					Sample (μl)	Reagents (μl)		Carrier (μl)	Total flow rate (ml min^{-1})
	E1	E2	E3	E4	E5		R_1	R_2		
Simultaneous injection of sample and reagents	On	On	On	Off	Off	200	80	80	–	6.0
Carrier injection	Off	Off	Off	On	Off	–	–	–	900	4.0

R_1: DMPD solution; R_2: Fe(III) solution.

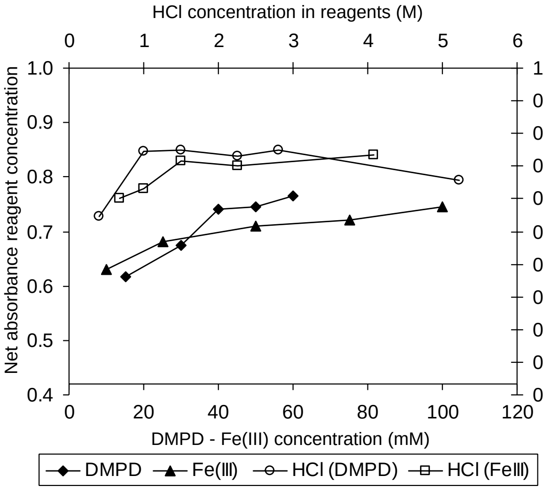

Fig. 2. Influence of the chemical variables on the recorded analytical signals. Sulfide concentration: 1.6 mg l^{-1} and DMPD: *N,N*-dimethyl-*p*-phenylenediamine monohydrochloride (See text for further details).

influence of DMPD concentration was evaluated over the range 15–60 mM in a 2.25 M HCl medium, maintaining Fe(III) concentration fixed at 100 mM. According to Fig. 2, improved sensitivity was obtained above 40 mM of the coupling amine. The effect of oxidizing reagent (prepared in a 2.25 M HCl medium) on the analytical signal was also studied over the range 10–100 mM, fixing the DMPD concentration at the 50 mM level. As observed in Fig. 2, the larger the Fe(III) concentration the higher the yields of Fischer's reactions are attained. However, concentrations above 60 mM Fe(III) should be avoided due to the deterioration of the analytical performance as a consequence of the severe influence of Schlieren effect interferences [18–22].

The dependence of the acidity of the solutions on the color developing reaction was also investigated. No significant differences in terms of limit of detection and repeatabilities were found within the interval 1.4–3.0 M HCl for both reagents (Fig. 2). Thus, the final composition of the chromogenic agents was set at 50 mM DMPD in 1.5 M HCl and 60 mM Fe(III) in 1.8 M HCl for subsequent experiments.

The weak point of LED-based spectrophotometric flowing stream procedures with liquid phase measurements is the inability to compensate the artifact signals resulting from the Schlieren effect by multiwavelength detection. Yet, refractive index changes at the front edge of the transient peak could be reduced to a large extent in the described flow system with the accurate selection of the composition of the carrier solution. Therefore, the carrier stream is recommended to be composed of 0.7 M HCl in order to match the acid concentration of the MB containing zone detected by the spectrophotometer.

3.3. *Investigation of flow injection parameters*

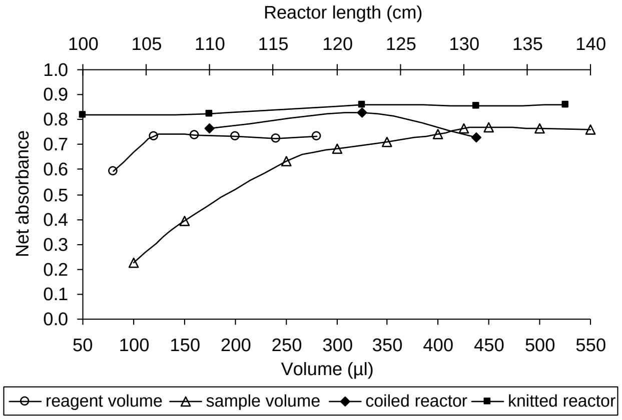

Fig. 3. Investigation of different injection modalities (single or sandwich-type multicommuted) sample and reagent volumes and reactor configurations (coiled or knitted) and effect on the analytical performance. Working conditions: 1.6 mg l^{-1} sulfide; 50 mM DMPD in 1.5 M HCl and 60 mM Fe(III) in 1.8 M HCl.

Under the optimized chemical conditions, the effect of flow injection parameters such as sample/reagent volumes and flow rates on the performance of the MSFIA methodology was tested using a sulfide concentration of 1.6 mg l^{-1}. A critical parameter to take into consideration is the sample volume, since according with Fig. 3, a 4-fold increase of peak height values was obtained by increasing the sample volume from 100 to 450 μl. Aiming to avoid excessive sample consumption without worsening the dynamic working range, a sample volume of 450 μl was thus selected for the sandwich-based procedure.

It should be stressed that a noteworthy feature of the multisyringe piston pump module is the wide range of flow rates available for a particular set of syringes. The injection of metered volumes of sample at flow rates ranging one order of magnitude (namely, from 0.9 to 9.0 ml min^{-1}) did not result in significant sensitivity differences, yet appreciable lower precision in the handled volumes is encountered above 6.0 ml min^{-1}. Similar behavior is found for the carrier dispensing rate. In order to ensure a suitable analytical throughput for monitoring purposes, sample and carrier flow rates of 6.0 and 4.0 ml min^{-1}, respectively, were used in both injection procedures.

Aiming to improve the reaction conditions the length and type of the reaction coil was also tested. Knitted and coiled reactors with lengths ranging from 100 to 180 cm were assayed. As shown in Fig. 3, knitted reactors provided better analytical performance as a consequence of the contribution of secondary toroidal flows [23] which improved the radial mixing between segments as well as reduced the axial dispersion of the generated MB species.

Taking into account the fast kinetics of reactions involved in Fischer's method, a 122 cm long knitted reactor, which matches the sizes of the injected zones, was chosen for further assays.

3.3.1. *Sandwich injection*

Two special benefits can be obtained from the application of the multicommuted sandwich injection technique

Table 3
Influence of the injection modality and reactor configuration on the recorded analytical signals

	Sample segment volume (μl)	Sample total volume (μl)	Reagents total volume (μl)	Knitted reactor		Coiled reactor	
				Net absorbance	Signal decrease (%)	Net absorbance	Signal decrease (%)
S/S+R/S	200–200–200	600	160	0.512±0.007	0	0.444±0.013	13.2
	125–200–125	450	160	0.512±0.009	0	0.437±0.008	14.6
	60–200–60	320	160	0.477±0.007	6.8	0.418±0.010	18.4
S+R	0–200–0	200	160	0.429±0.011	16.1	0.395±0.016	22.9

Sulfide concentration: 1.5 mg l^{-1}; reagents total volume: same volumes of DMPD and Fe(III); S: sample; R: reagents.

described in the Experimental section with respect to a single sample and reagent injection for a fixed sample volume (namely 450 μl). On one hand, a better interdispersion between zones is warranted, on the other hand more than 2-fold reagent saving (from 360 to 160 μl) may be accomplished, while also attaining a 15% increase of peak height.

In addition, the sample plug volumes of the sandwich-based injection were optimized. As can be observed in Table 3, the diminution of the size of the border sample slugs causes a decrease of the analytical signal. This result indicates that the edged sample plugs are interdispersed downstream with reagents, thus, improving the mixing profile. A 125–200–125 μl of sample scheme was selected in order to guarantee the highest peak height and the lowest sample consumption. Moreover, other noteworthy feature that can be observed in Table 3 is the best analytical signal obtained in all cases by using the knitted reactor.

It should be also highlighted that the sensitivity of the optimized multicommutation sandwich-based injection technique is considerably improved in comparison to that reported in previous flowing stream techniques involving the methylene blue methodology (see Table 7).

3.3.2. Multiple injection

Two advantages can be obtained from the application of the multicommuted multiple injection technique described in the Experimental section with respect to a sandwich modality. On one hand, a better injection frequency is attained (injections, from 45 to 80 h^{-1}), on the other hand more than 2-fold sample saving (from 450 to 200 μl) may be accomplished according to Fig. 3, in spite of a 61% decrease of sensitivity. Thus, the multiple injection modality is recommended if a high sampling frequency is required.

3.4. Analytical features of the optimized method

The analytical figures of merit of both optimized MSFIA procedures with multicommuted injection are listed in Table 4. A linear working range within one order the magnitude, which agrees with previous results [17,24] was obtained. The detection limit was assessed from three times the standard deviation of the blank solution and the repeatability from 10 independent and consecutive measurements (with three injection each one) at the 1 mg l^{-1} level (1.38 and 1.44% for sandwich and multiple injection, respectively). The robustness of both automatic methods was verified from calibration plots constructed in ten different working days from a freshly prepared sulfide stock solution ($n = 10$). Thus, the coefficients of variation were 3.04% for sandwich and 1.11% for multiple injection modalities. This result together with the high analytical throughput available and the extremely stable baseline recorded reveal the suitability of the designed system to be implemented in monitoring stations. Besides, it should be emphasized that the sensitivity of the sandwich technique MSFIA analyser calculated according to the IUPAC criterion [25] is more than 2-fold better than that described in previous flowing stream methods [16–24].

3.5. Evaluation of interferences

The species tested as potential interferents in the determination of sulfide under dynamic conditions are those most frequently present in anoxic aquatic environments, i.e. reduced forms such as ammonium, and those typically found in wastewater. Additionally, the influence of seawater electrolytes on the analytical signal was also investigated. A species was considered interferent when its concentration affected more than 10% the absorbance readings.

Table 5 shows the high tolerance levels to common ionic species found in environmental waters. The most severe interferences are obviously caused by metal ions able to form

Table 4
Figures of merit of the photometric MSFIA approaches for sulfide monitoring

Analytical Parameter	Sandwich technique	Multiple injection modality
Detection limit (mg l^{-1})	0.09	0.15
Slope of calibration graph[a]	0.43 ± 0.02	0.17 ± 0.01
Regression coefficient[a]	0.9991 ± 0.0005	0.9988 ± 0.0003
Linear dynamic range (mg l^{-1})	0.2–2.0	0.5–5.0
R.S.D. (%), (1 mg l^{-1}) ($n = 10$)	1.38	1.44
Injection frequency (h^{-1})	45	80
Sampling frequency (h^{-1})[b]	13	27

[a] Average ± standard deviation ($n = 10$).

[b] Sampling frequency is calculated considering three replicates per sample and the rinsing of the sample aspiration tubing.

Table 5
Investigation of potential interferents in the MSFIA determination of sulfide at 1 mg l^{-1} concentration

Interferent/sulfide ratio	Foreign species
100[a]	Al^{3+}, Ca^{2+}, Cd^{2+}, CO_3^{2-}, EDTA, K^+, Mg^{2+}, Na^+, NO_3^-, PO_4^{3-}, SO_4^{2-}, Zn^{2+}
80	NH_4^+
10	NO_2^-
1	Mn^{2+}, Pb^{2+}
0.5	Hg^{2+}
<0.05	Cu^{2+}

[a] Maximum concentration assayed.

insoluble salts or inorganic species involved in redox reactions. Nevertheless, the tolerance to some oxidants, such as nitrite, is 10-fold improved in the proposed MSFIA configuration in comparison with previous reverse-FIA systems [17].

3.6. Analysis of real samples

The applicability of the optimized automatic analyzer was evaluated through the determination of the target species in various water matrices (namely, mineral, tap, ground, seawater and wastewater). The possible existence of multiplicative matrix interferences was also assessed via sample spiking at the 1 mg l^{-1} level.

Samples were immediately analysed after collection to prevent oxidation of the target species using both the MSFIA method and that recommended by APHA-AWWA-WPCF [11]. Recoveries ranging from 96 to 104% were obtained for the entire set of samples analysed indicating that no significant losses of sulfide occurred during the analyses. These results also proved the inexistence of multiplicative matrix interferences, the MSFIA analyzer being thus suitable for monitoring sulfide in wastewaters. Good agreement was found between the developed MSFIA system and the batch standard method when applied to the determination of the target analyte in a wastewater sample collected at the clarifying step (flocculation with Fe(III) salts). Wastewater sample, collected in a wastewater treatment plant (EDAR-1, Palma de Mallorca), had the following chemical composition expressed in mg l^{-1}: BOD5 291 and 77, COD 737 and 271, suspended solids 457 and 138 for inlet (pH 7.7) and outlet (pH 7.2), respectively. The results of the recovery assay applied to the entire set of analysed water samples are presented in Table 6.

3.7. Comparison of the analytical features with those of previous flowing stream methods

In this section, MSFIA approach is compared with different flow techniques described in the literature, such as flow injection analysis (FIA), reverse flow injection analysis (r-FIA), monosegmented flow analysis (MSFA) and sequential injection analysis (SIA). The analytical figures of merit of each method are summarized in Table 7. As can be seen, both injection modalities of MSFIA provide extremely low variation coefficients due to the accuracy of the sample and reagent volumes handled by the multisyringe pump as well as the stability of the light source. Besides, MSFIA-sandwich technique gives the best sensitivity in comparison to other flow injection methods, and despite the periodical filling-up of syringes with solutions from reservoirs, the sample frequency is comparable (or even better) to that reported in flow injection systems. Improvement of sample throughput is readily attainable via the MSFIA-multiple injection approach. The sample volume/injection showed in Table 7 is referred to a simple injection without considering the sample volume required to fill the holding coil or for starting data acquisition. Thus, it is important to point out that while FIA and r-FIA use a continuous sample flow, SIA and MS-

Table 6
Multisyringe flow injection determination of sulfide in real samples and spiked water matrices

Sample	Added (mg l^{-1})	MSFIA-sandwich technique		Standard APHA-method	
		Found (mg l^{-1})	Recovery (%)	Found (mg l^{-1})	Recovery (%)
Mineral water	0	N.D.		N.D.	
	1	0.98 ± 0.01	98	1.00 ± 0.01	100
Seawater	0	N.D.		N.D.	
	1	0.97 ± 0.01	97	0.95 ± 0.01	95
Tap water	0	N.D.		N.D.	
	1	1.01 ± 0.01	101	1.00 ± 0.01	100
Groundwater	0	N.D.		N.D.	
	1	0.96 ± 0.01	96	0.98 ± 0.01	98
Wastewater[a] outlet	0	N.D.		N.D.	
	1	1.04 ± 0.01	104	1.01 ± 0.01	101
Wastewater[a] clarifying step[b]	0	5.96 ± 0.08		5.60 ± 0.17	
	1	6.93 ± 0.01	97	6.62 ± 0.15	102

The results are expressed as the mean of four replicates ± confidence interval at the 0.05 significance level. N.D.: Not detected.

[a] Flocculated wastewater.

[b] The sample was 5-fold diluted prior analysis.

Table 7
Comparison of analytical features of different flowing stream techniques for the spectrophotometric determination of sulfide using the MB method

Method	Slope	R.S.D. (%)	Throughput injection (h^{-1})	Linear range (mg l^{-1})	Detection limit (mg l^{-1})	HCl carrier (mg/injection)	HCl reagents (mg/injection)	Sample injected volume (ml)	Waste (ml h^{-1})	Reference
FIA	0.005	–	210	1–45	<1	–	1110	0.065	435	[16]
FIA	–	2.10	30	0–2.88	0.001	–	197	1.20	108	[12]
Reverse-FIA	0.189	0.71	48	0.5–5	<0.5	–	666	2.63	–	[17]
SIA	0.136	4.00	60	0.05–2	0.04	535	48	0.15	213	[26]
SI-MSFA	0.132	5.20	38	0.17–1	0.04	254	34	0.12	129	[24]
MSFIA-sandwich	0.430	1.38	45	0.2–2	0.09	191	29	0.45	121	This work
MSFIA-multiple injection	0.167	1.44	80	0.5–5	0.15	69	29	0.20	115	This work

FIA techniques only dispense sample when it is needed. The injection of well defined zones of sample and reagents through the activation of the solenoid valves warrants minimum waste generation (3.6-fold reduction in comparison with typical FI-manifolds) and, alike SIA procedures, more than 10-fold saving of aggressive reagents. The straightforward configuration of both the flow network and the optical detector, is also worth mentioning.

4. Conclusions

In this paper, the potentials of the multisyringe flow injection technique in combination with multicommutation schemes have been demonstrated and exploited for the determination of sulfide in environmental and wastewaters. A fully automated, software-controlled flow system with robustness and reliability assessed via sample spiking and exploration of the day-to-day reproducibility has been developed. As a consequence of the versatility of both MSFIA manifold and software, an automated method with either high sensitivity or improved injection frequency can be attained within a single manifold. The recoveries obtained, ranging from 96 to 104%, proved that no significant losses of sulfide occurred during the automatic analyses.

The miniaturized LED-based optical system designed makes it especially suitable for real-time monitoring schemes. Moreover, as opposed to precedent flow techniques, MSFIA affords several additional advantages, such as compactness, improved versatility, and lower sample/reagent consumption, which in turn leads to a minimum waste generation.

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References

[1] V. Cerdà, J.M. Estela, R. Forteza, A.CladeraE. Becerra, P. Altimira, P. Sitjar, Talanta 50 (1999) 695.
[2] F. Albertús, B. Horstkotte, A. Cladera, V. Cerdà, Analyst 124 (1999) 1373.
[3] B.F. Reis, M.F. Giné, E.A.G. Zagatto, J.L.F.C. Lima, R.A. Lapa, Anal. Chim. Acta 293 (1994) 129.
[4] P.B. Martelli, B.F. Reis, E.A.M. Kronka, H. Bergamin Fo, M. Korn, E.A.G. Zagatto, J.L.F.C. Lima, A.N. Araujo, Anal. Chim. Acta 308 (1995) 397.
[5] A.N. Araújo, J.L.F.C. Lima, B.F. Reis, E.A.G. Zagatto, Anal. Chim. Acta 310 (1995) 447.
[6] F.R.P. Rocha, B.F. Reis, Anal. Chim. Acta 409 (2000) 227.
[7] F.R.P. Rocha, P.B. Martelli, B.F. Reis, Anal. Chim. Acta 438 (2001) 11.
[8] F.R.P. Rocha, B.F. Reis, E.A.G. Zagatto, J.L.F.C. Lima, R.A.S. Lapa, J.L.M. Santos, Anal. Chim. Acta 468 (2002) 119.
[9] M. Miró, J.M. Estela, V. Cerdà, TrAC, Trends Anal. Chem. 21 (2002) 199.
[10] U. Förstner, in: W. Salomons, W. Stigliani (Eds.), Biogeodynamics of Pollutants in Soils and Sediments, Springer, Germany, 1995, p. 247.
[11] APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 17th ed., 1992.
[12] V. Kuban, P.K. Dasgupta, J.N. Marx, Anal. Chem. 64 (1992) 36.
[13] N.S. Lawrence, J. Davis, R.G. Compton, Talanta 52 (2000) 771.
[14] E. Becerra, A. Cladera, V. Cerdà, Lab. Rob. Autom. 11 (1999) 131.
[15] R.J. Casella, L.S. Teixeira, S. Garrigues, A.C. Spinola, E. Santelli, M. De la Guardia, Analyst 125 (2000) 1835.
[16] D.J. Leggett, N.H. Chen, D.S. Mahadevappa, Anal. Chim. Acta 128 (1981) 163.
[17] A. Ríos, M.D. Luque de Castro, M. Valcárcel, Analyst 109 (1984) 1487.
[18] E.A.G. Zagatto, M.A.Z. Arruda, A.O. Jacintho, I.L. Mattos, Anal. Chim. Acta 234 (1990) 153.
[19] V.O. Brito, I.M. Raimundo Jr., Anal. Chim. Acta 371 (1998) 317.
[20] H. Liu, P.K. Dasgupta, Anal. Chim. Acta 289 (1994) 347.
[21] I.D. McKelvie, D.M.W. Peat, G.P. Matthews, P.J. Worsfold, Anal. Chim. Acta 351 (1997) 265.
[22] R.A.S. Lapa, J.L.F.C. Lima, B.F. Reis, J.L.M. Santos, E.A.G. Zagatto, Anal. Chim. Acta 366 (1998) 209.
[23] Z.L. Fang, Flow Injection Separation and Preconcentration, VCH, Weinheim, Germany, 1993, p. 169 (Chapter 7).
[24] M.S.P. Silva, C.X. Galhardo, J.C. Masini, Talanta 60 (2003) 45.
[25] G.L. Long, J.D. Winefordner, Anal. Chem. 55 (1983) 712 A.
[26] M.S.P. Silva, I.S. da Silva, G. Abate, J.C. Masini, Talanta 53 (2001) 843.