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Interfacing in-line gas-diffusion separation with optrode sorptive preconcentration exploiting multisyringe flow injection analysis

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Abstract

An automatic multisyringe flow injection analysis (MSFIA) system coupling a flow-through optical fiber diffuse reflectance sensor with in-line gas-diffusion (GD) separation is proposed for the isolation, preconcentration and determination of traces of volatile and gas-evolving compounds in samples containing suspended solids, with no need for any preliminary batch sample treatment. The flowing methodology overcomes the lost of sensitivity of the in-line separation technique, when performed in a uni-directional continuous-flow mode, through the implementation of disk-based solid-phase extraction schemes. The high selectivity and sensitivity, the low reagent consumption and the miniaturization of the whole assembly are the outstanding features of the automated set-up. The proposed combination of techniques for separation, flow analysis, preconcentration and detection was applied satisfactorily to sulfide determination in environmental complex matrixes. The method based on multicommutation flow analysis involves the stripping of the analyte as hydrogen sulfide from the donor channel of the GD-module into an alkaline receiver segment, whereupon the enriched plug merges with well-defined zones of the chormogenic reagents (viz., *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) and Fe(III)). The in-line generated methylene blue dye is subsequently delivered downstream to the dedicated optrode cell furnished with a C_{18} disk, while recording continuously the diffuse reflectance spectrum of the pre-concentrated compound. This procedure provides a linear working range of 20–500 $\mu g\,l^{-1}$ sulfide with a relative standard deviation of 2.2% ($n = 10$) at the 200 $\mu g\,l^{-1}$ level, and a detection limit of 1.3 $\mu g\,l^{-1}$.

Keywords: Gas-diffusion; Optrode; Preconcentration; Multisyringe flow injection analysis (MSFIA); Sulphide; Waters

1. Introduction

The combination of techniques for flow drive, separation, preconcentration and detection, has opened new pathways in analytical chemistry for the development of entirely automated assemblies adaptable to real-time monitoring schemes.

Separation techniques play a crucial role in the application of analytical methods with major aims of analyte isolation and removal of interfering compounds. Gas diffusion in combination with flow systems facilitates the selective determination of volatile compounds in a totally enclosed manifold [1–3].

Isolation with concomitant enrichment of target species is accomplished with solid-phase extraction procedures that have been consolidated as valuable sample pre-treatment strategies [4]. The direct optosensing at solid surfaces avoids the disadvantage of the traditional sorbent extraction, involving the elution of the retained species to perform the optical detection in the eluate phase, of partial loss of the preconcentration capabilities gained during the sorption step. It is based on direct measurement of the light attenuation of the reactive surface following the sorptive preconcentration of the analyte properly derivatized [5–8]. However, to the best of our knowledge, the hyphenation of flow-through GD with sorptive optrode preconcentration and detection has not yet been reported.

Regarding recent advances in automation in flow systems, the multisyringe flow injection analysis (MSFIA) approach is worth mentioning. It should be regarded as a powerful tool for interfacing in-line GD separation with solid-phase optosensing with inherent potential for appropriate microfluidic handling of solutions [9–11]. The main objective of this novel flowing methodology is to obtain maximum benefit of the ruggedness and flexibility of sequential injection schemes using syringe pumps as liquid drivers. The coupling of four soldered piston pumps with solenoid valves facilitates a multitude of different injection

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modalities (namely, multicommutation protocols, splitting techniques, sandwich schemes and hydrodynamic injections) to be readily performed [11–13]. Furthermore, the three-way commutation valves placed at the head of each syringe enable the injection of minute, well-defined volumes of sample and reagents according to the timing schedule of the analytical protocol. Since reagents are dispensed back to the respective reservoirs when not needed, waste generation is more than 10-times reduced in comparison to common flow injection procedures.

The combination of techniques herein reported enables the selective, sensitive and automated determination of volatile species by direct detection or following previous derivatization reactions. Sulfide has been selected as a model analyte to assess the potential of the MSFIA-GD-optosensor coupling.

Sulfide is one of the most important parameters to monitor in water matrices as a consequence of its high toxicity for aquatic organisms [14]. Hydrogen sulfide is found in natural and wastewaters as a result of the decomposition of organic matter and the bacteria-mediated reduction of sulfate. In addition, hydrogen sulfide is frequently used as a reagent or generated as a by-product in industrial processes. Due to the increasing environmental concern for chemical discharges, the sulfide content in industrial effluents must be continuously controlled. Nevertheless, the classical methods for sulfide monitoring in complex environmental matrices, such as seawaters and wastewaters, are time-consuming and require preliminary sample treatment procedures (e.g., flocculation of suspended solids) to guarantee accurate results [15]. Besides, although sulfide is currently regarded, even at low concentrations, as a key parameter to assess the environmental quality of a given system, conventional batch-wise spectrophotometric methodologies lack of appropriate sensitivity, whereby new approaches capable of monitoring trace level concentrations are called for.

The aim of this work is thus to demonstrate the capabilities of multisyringe flow injection analysis (MSFIA) with gas-diffusion separation coupled to sorptive flow-through diffuse reflectance spectroscopy for monitoring trace levels of sulfide in complex environmental matrices, even those containing suspended solids, without requiring any additional batch preliminary treatment. The hyphenated method is based on the analyte release as hydrogen sulfide from the untreated sample into the recipient channel of the GD-module, derivatization according to Fischer's reaction [16], and integrated in-line preconcentration and detection of the generated methylene blue (MB) using a custom-built flow-through disk-based diffuse reflectance optosensor.

2. Experimental

2.1. Reagents and membranes

All reagents were of analytical grade quality 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 *N,N*-dimethyl-*p*-phenylenediamine monohydrochloride (DMPD, Sigma) in approximately 50 ml of water, to which 10 ml of 5 M HCl are added before diluting to 100 ml with water. A 3 mM DMPD working solution was prepared by suitable dilution of the stock solution with 0.5 M HCl. The oxidizing reagent consisted of 9.64 g of $NH_4Fe(SO_4)_2{\cdot}12H_2O$ (Scharlau, Barcelona, Spain) dissolved in a mixture of 50 ml of water and 10 ml of 5 M HCl and made up to 100 ml with water. A 3 mM Fe(III) solution was prepared by proper dilution of the stock with 0.5 M HCl.

A 0.14 M HCl solution prepared from concentrated hydrochloric acid (Scharlau) was used as a carrier stream. The alkaline acceptor consisted of a 10^{-3} M NaOH solution. Solutions of 80% (v/v) methanol–0.01 M HCl and 80% (v/v) methanol–water were used as eluting solutions, the latter one also ensured appropriate membrane conditioning. Prior to use, these reagents were degassed for 10 min by means of an ultrasonic bath.

The sulfide stock standard solution (ca. 1000 mg l^{-1}) was prepared daily by dissolving 0.75 g of $Na_2S{\cdot}9H_2O$ 95% (Panreac, Barcelona, Spain) in 50 ml of a 0.05 M NaOH solution and diluting to 100 ml with water. The final solution was daily-standardized iodimetrically [15] and working standard solutions (20–500 μg l^{-1}) were prepared by suitable dilution of the stock with 0.025 M NaOH.

A Durapore (polyvinylidene fluoride, PVDF) membrane with 0.22 μm pore size and 125 μm thickness purchased from Millipore (Bedford, MA, USA) was employed as a hydrophobic barrier for physical separation of donor and acceptor streams.

Octadecyl-bonded silica gel (C_{18}) Empore disks of 0.5 mm thickness purchased from 3 M (St. Paul, MN, USA) were cut into small membrane disks with a diameter of 9 mm to be used in the laboratory-built optrode flow-cell.

2.2. Flow system, optical detector and software

The multisyringe flow injection manifold coupling a GD unit with a flow-through optical fiber diffuse reflectance sensor for the separation, derivatization, and integrated sorption and detection of sulfide is shown in Fig. 1. Two multisyringe piston pump modules (Crison, Barcelona, Spain) were used as liquid drivers. One of them was equipped with four syringes (Hamilton, Switzerland) S1–S4 of 1, 1, 5 and 5 ml, respectively, whilst the other multisyringe module was furnished with the syringes S5–S8 of 1, 10, 10 and 5 ml, respectively. Each syringe has a three-way solenoid valve (N-Research, Caldwell, NJ, USA) at the head (E1–E8), which facilitate the application of multicommutation schemes. The automatic control of the flow device was made via PC through an RS232C interface. The multisyringe module also comprised two additional discrete solenoid valves (model MTV-3-N 1/4 UKG, Takasago, Japan) (V1 and V2), which were crucial for the performance of the analytical protocol. V1 directed the sample zone from the autosampler (Crison) to the holding coil (HC), whereupon the flow was reversed and the aspirated plug was delivered to the GD module. V2 delivered solely the sample/reagents plugs through the extraction disk whenever the preconcentration step was initiated. Hence, valve V2 allowed the performance of the initialization operations and

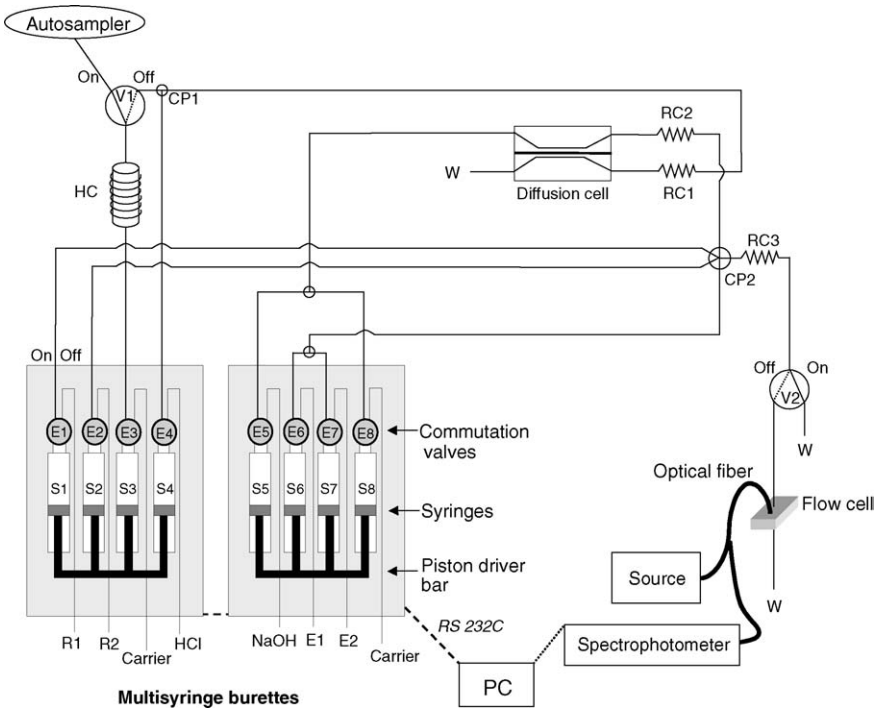

Fig. 1. Schematic diagram of the gas-diffusion multisyringe flow injection set-up with integrated flow-through optical fiber diffuse reflectance sensor. R1: 3 mM DMPD; R2: 3 mM Fe(III); E1: 80% (v/v) methanol–water; E2: 80% (v/v) methanol–0.01 M HCl; carrier: 0.14 M HCl; CP: confluence point; HC: holding coil; RC: reaction coil, RC1: 130 cm, RC2: 200 cm, RC3: 200 cm.

the rinsing of the flow lines without dispensing any solution through the sorptive membrane.

Three- and five-way connectors were made from PMMA, while the holding coil, reaction coils and connections were made from PTFE tubing of 0.8 mm i.d., excepting the holding coil tubing which was of 1.5 mm i.d. The length of HC and reaction coils RC1, RC2 and RC3 were 400, 130, 200 and 200 cm, respectively. The reaction coils were constructed by interlacing the PTFE tubing in knots of ca. 5 mm diameter.

The membrane-based separation unit consisted of two TEFZEL blocks with S-shaped semi-tubular grooves providing an effective transfer area of 1.0 cm^2 and an inner volume of 25 μl each. The groove in one plate perfectly matched with its mirror image.

The laboratory-made flow-through cell as assembled for disk based solid-phase extraction and reflectance measurements is described elsewhere [17]. Briefly, it comprises two PEEK blocks, one of them being machined to accommodate the main leg of the optical fiber, a window obtained from UV-cuvette Plastibrand (Wertheim, Germany) to protect the fiber from aggressive reagents, a thin PTFE spacer, and holders.

The miniaturized optical detector involved a diode-array USB 2000 spectrophotometer (Ocean Optics, Dunedin, FL) and a laser light emitting diode (red diode, 75 mA maximum intensity, spectral band of 620–700 nm, Sciware, Palma de Mallorca, Spain) with a power supply of adjustable intensity (Sciware). The light source was connected to the purpose-made flow-through cell, via one leg of a reflection bifurcated optical fiber (Ocean Optics) with a core diameter of 400 μm. The reflected radiation was carried back to the detector through the other leg of the optical fiber with a core diameter of 400 μm. The analytical wavelength selected for monitoring the MB dye was 666 nm.

Instrumental control, acquisition of diffuse reflectance data, signal evaluation and determination of analyte concentrations with integrated calibration were performed using the software package AutoAnalysis 5.0[1] [18] (Sciware). The developed software based on dynamic link libraries (DLLs) at 32 bits comprises a single and versatile protocol, which allows the implementation of specific and individual DLLs according to 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 devices, diode-array photometer and autosampler.

2.3. Multisyringe flow injection protocol

The automated protocol for MSFIA-GD isolation and solid-phase optosensing of sulfide is detailed in Table 1, and summarized as follows:

1. Initially, the syringes are filled with solutions from the respective reservoirs at 1.0 ml min^{-1} (for S1) with heads 1-Off 2-Off 3-On 4-Off 5-Off 6-Off 7-Off 8-Off and valves 1-On 2-Off. The HC is simultaneously loaded with 5.0 ml of sample via activation of V1.
2. In order to initialize the system, sample and acid solution zones are delivered to confluence point CP1 (steps 1 and 2 in Table 1).

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

Table 1
Multicommutated protocol for the flow-through gas-diffusion (GD) separation and optrode preconcentration of sulfide using multisyringe flow injection analysis (MSFIA)

Steps	Solenoid valve schedule									Donor stream		Acceptor stream (ml)	Reagents		Carrier (ml)	Eluent		Flow rate for S1 (ml min^{-1})
	E1	E2	E3	E4	E5	E6	E7	E8	V1	V2	Sample (ml)	1.5 M HCl (ml)		DMPD (ml)	Fe(III) (ml)		MeOH 80% (ml)	MeOH 80%–0.01 M HCl (ml)	
System initialization																			
(1) Delivery of sample to CP1*	Off	Off	On	Off	Off	Off	Off	Off	Off	On	0.045	–	–	–	–	–	–	–	2
(2) Delivery of 1.5 M HCl to CP1*	Off	Off	Off	On	Off	Off	Off	Off	Off	On	–	0.100	–	–	–	–	–	–	2
In-line separation by gas diffusion																			
(3) Merging sample with acid towards the GD-module*	Off	Off	On	On	Off	Off	Off	Off	Off	On	0.330	0.330	–	–	–	–	–	–	0.50
(4) Receiver solution is dispensed to the GD-module	Off	Off	Off	Off	On	Off	Off	Off	Off	On	–	–	0.230	–	–	–	–	–	0.50
(5) GD separation	Off	Off	On	On	On	Off	Off	Off	Off	On	4.625	4.625	0.991	–	–	–	–	–	0.18
MB formation																			
(6) Development of Fischer's reactions	On	On	Off	Off	Off	Off	Off	On	Off	Off	–	–	–	0.200	0.200	1	–	–	0.18
Preconcentration on the C_{18} extraction disk																			
(7) Recording the transient analytical signals	Off	Off	Off	Off	Off	Off	Off	On	Off	Off	–	–	–	–	–	2	–	–	0.18
Elution and regeneration																			
(8) Desorbing the reaction product and conditioning the extraction disk*	Off	Off	Off	Off	Off	On	Off	Off	Off	Off	–	–	–	–	–	–	1	–	0.50
	Off	Off	Off	Off	Off	Off	On	Off	Off	Off	–	–	–	–	–	–	–	1	0.50

CP1: confluence point #1. S1: syringe #1 (1 ml). E1–E8 and V1–V2: solenoid valves.
* Multi-commutation step.

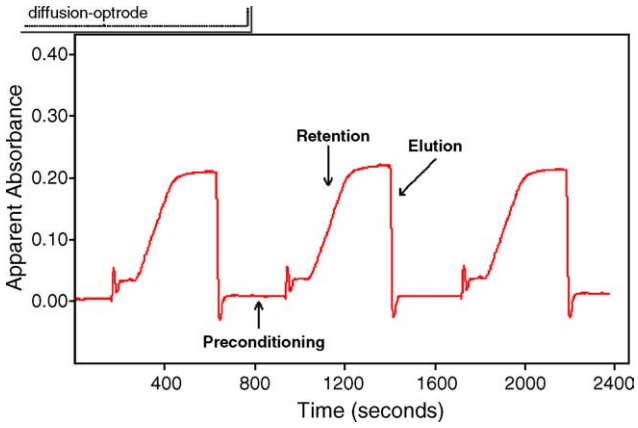

Fig. 2. Illustration of the transient analytical signal for 100 μg l^{-1} sulfide showing the various analytical steps involved in the optrode determination.

3. After directing the various solutions to the inlets of the GD-module (steps 3 and 4), the receiver zone and the acidic feeding stream are simultaneously dispensed in a counter-current continuous-flow fashion at a total flow rate of 1.8 and 0.18 ml min^{-1} for donor and acceptor, respectively (step 5). At this step, the hydrogen sulfide formed is stripped from the feeding solution and trapped into the acceptor solution as sulfide. The enriched zone is stacked in the reaction coil (RC2).
4. The diode-array spectrophotometer starts data acquisition at a reading frequency of 1 Hz.
5. Afterwards, diffusate plug and reagent segments merge simultaneously in RC3, taking place Fischer's reactions prior to reach the optrode cell (step 6).
6. In order to record the transient analytical signals, the multisyringes burettes are programmed to propel a metered carrier volume in RC3 (step 7).
7. The on-line formed MB dye is pre-concentrated on the C_{18} extraction disk, thereby attaining a minimum diffuse reflectance value (maximum apparent absorbance—plateau level) when the entire sample plug has flowed through the sorptive membrane.
8. After recording the plateau level, the eluting solutions (namely, 80% (v/v) methanol–0.01 M HCl and 80% (v/v) methanol–water) are sequentially injected for desorbing the reaction product and conditioning the extraction disk before starting a new analytical cycle (step 8). The difference between baseline and plateau level is taken as analytical signal. The transient optrode readouts are schematically shown in Fig. 2.
9. The operational sequence is repeated three-times from step 3.

3. Results and discussion

3.1. Potential of the MSFIA-GD-optrode hyphenation

Gas-diffusion [19] and pervaporation [20] are well consolidated membrane-based flow-through techniques for the separation of volatiles and gas-evolving species from solid and liquid

matrices. Their main goal is to increase the selectivity of analytical methodologies by preventing interfering sample components to reach the detection instrument: ionic compounds are totally excluded and colored and cloudy samples can generally be analyzed, even with spectrophotometric methods, as facilitated in monitoring stations by direct injection/aspiration from the sampling site.

In addition, several researchers have recognized the preconcentration capabilities of gas-diffusion assemblies by the implementation of stopped-flow or re-circulation schemes in a recipient closed-loop configuration [21,22]. Yet, the moderate enrichment factors attained and the high frequency of maintenance for time-based procedures owing to the periodical recalibration of the flow system detracted from their broad appeal. Both shortcomings can be elegantly circumvented by exploiting the robustness and versatility of the liquid drivers of the MSFIA set-ups. Not the least, by the reproducible and accurate manipulation of sample and reagent zones in the conduits of the flow network, and the selection of the flow rate for both donor and acceptor phases at will. A wealth of in-line separation modes arises from the MSFIA-GD coupling either in a stagnant or flow fashion. Hence, halted receiver plugs can be readily combined with software-controlled continuous, plug-in or backward–forward flow approaches for the sample medium.

Enhanced GD efficiency is accomplished by exhaustive diffusion procedures capitalized on stopping the donor phase while pumping continuously the receiver stream. Obviously, the moving acceptor phase gives rise to dilution that can be, however, overcome by preconcentration of the diffused analytes via a trace enrichment column. In fact, solid traps alike sorbent containing microcartridges are the essential component of the so-called membrane extraction with sorbent interface (MESI) [23] commonly used to retain volatile organics prior to capillary GC determinations. In this paper, we describe for the first time a supplementary approach for integrating preconcentration and detection of released species based on disk-phase optrode measurements. The absence of detectable backpressure for long-term operational sequences, and the straightforward adjustment of the optical fiber to the sensing microzone make this alternative particularly attractive for trace level analysis. As discussed later, the MSFIA-GD-optical sensor hyphenation also admits the synchronous and continuous flow of sample and reagent segments on both sides of the membrane without deterioration of the analytical sensitivity whenever the feeding and recipient stream are delivered to the separation module at significantly different flow rate.

3.2. Configuration of the MSFIA-GD-optrode system

Amongst the different modes for GD stripping, namely, stopped-flow and continuous-flow, the latter procedure was selected to assess the potential of the hyphenated MSFIA method. Despite the further transformation of the analyte into a non-diffusible form in the recipient plug, the counter-current flow injection mode yielded a sensitivity improvement of 64% with respect to concurrent flow approaches.

The first option selected as an acceptor solution was the mixture of chromogenic reagents, namely, DMPD and Fe(III) in acidic medium, at concentrations of 25 and 60 mM, respectively, as being satisfactorily used earlier in flow-through GD separations [24]. Nevertheless, this receiver solution resulted inappropriate for the sorptive optosensor as a consequence of the saturation of the preconcentration membrane by the chromogenic reagents. The chemical and optical interferences were largely minimized by decreasing the concentration of derivatizing agents but at expenses of a dramatic decrease of Fisher's reaction yield. Hence, the GD-module was placed before the mixture of the analyte with the reagents, using an alkaline solution as acceptor stream.

The sorbent materials for the solid-phase preconcentration optosensing system were selected from commercially available extraction membranes. In a previous work, octadecyl-bonded silica gel (C_{18}), poly-styrenedivinylbenzene-copolymer (SDB-XC) and cation exchange-SR Empore 3M disks were evaluated as sorptive surfaces for sulfide preconcentration [17]. C_{18} membranes were selected as optrode active microzones due to the lower blank signals recorded and the facility for performing the conditioning/regeneration protocol. Moreover, the repeatability values for optrode assays involving C_{18} membranes were better than those obtained with the other disks.

3.3. Investigation of chemical variables

An indispensable condition for suitable performance of the in-line separation system for sulfide is the transformation of the analyte into a volatile compound by pH shift. pH adjustment was accomplished on-line using diluted hydrochloric acid. The reagent concentration was varied from 0.5 to 3.0 M HCl. The release of hydrogen sulfide was increased with increasing acid concentrations, up to 1.5 M HCl, which was adopted for subsequent MSFIA experiments.

The effect of the acceptor solution concentration was evaluated from 10^{-5} to 10^{-1} M NaOH. Maximum sensitivity was obtained within the range 10^{-3} to 10^{-2} M, the lowest concentration being selected for the remainder of the work.

The influence of several chemical variables (e.g., reagent concentrations and reagent acidity) affecting the performance of flow-through optical fiber diffuse spectroscopy was studied thoroughly elsewhere [17]. Accordingly, the final composition of the chromogenic agents was set at 3 mM DMPD in 0.5 M HCl and 3 mM Fe(III) in 0.5 M HCl. It should be noted that the concentration of hydrochloric acid used in the present work is much lower than that of previous flow-through or batch-wise methods, in which the acidity of the medium ranged from 1.0 to 6.0 M HCl [25–27].

A 0.14 M HCl solution was employed as a carrier stream to match the acid concentration of the MB zone; the baseline signal was, thus, kept at a negligible, constant level throughout the overall analysis cycle.

MB elution from C_{18} membranes was examined using methanol–water solutions. Incomplete removal of the sorbed MB species was observed with ratios <70% (v/v) methanol, whilst >80% (v/v) methanol could not be used because of the

generation of vapor bubbles in the flow system. Although 80% (v/v) methanol entirely eluted the MB dye from the reverse-phase disks, an additional regeneration solution containing 80% (v/v) methanol–0.01 M HCl was also delivered to the active surface to minimize the background noise, rendering improved detection limits.

3.4. Investigation of multisyringe flow injection parameters

The hydrodynamic parameters, which would most likely influence the analytical sensitivity, are the sample and reagent volumes, flow rates and the length and type of reactors.

The size of the sample zone delivered to the GD module was evaluated from 1 to 7 ml. Samples volumes above 5.0 ml did not cause significant sensitivity differences. Aiming to avoid excessive sample consumption without worsening neither the dynamic working range nor the detection limit, a sample volume of 5.0 ml was selected for the GD procedure. In continuous-flow approaches, this parameter along the donor/acceptor flow rate ratio defines the length of the acceptor segment to be preconcentrated.

The effect of the flow rate of both the feeding and acceptor streams on the mass transfer efficiency was also evaluated for the particular set of liquid drivers. To decrease the thickness of the diffusional layer for the target species but at the same time to fulfill the kinetic requirements of the dynamic GD process, the donor solution was pumped continuously at a 10-fold higher rate than the receiver stream, i.e., 1.8 ml min^{-1}.

The influence of the size of reagent zones on the development of the derivatization reaction was studied from 100 to 300 μl for a fixed receiver plug (viz., 1.0 ml). Since the diffusate and reagent plugs are accurately metered via software programming and injected simultaneously from the confluence CP2 (see Fig. 1), merely 200 μl of DMPD and 200 μl of oxidizing reagent were required to match the length of the enriched sample plug at the applied flow rates. As a result, a remarkable reagent saving in comparison with conventional flow injection methods based on the continuous flowing of reagent streams is achieved.

Knotted reactors were preferred over straight and coiled tubing as a consequence of the major contribution of secondary toroidal flows [28], which enhance the radial mixing between segments as well as decrease the axial dispersion of both the analyte containing receiver slug and the generated MB. Aiming to improve the reaction yields under dynamic conditions, the length of the reaction coil RC3 was varied from 150 to 350 cm. The shortest reactors provided insufficient contact time between segments while reactors longer than 200 cm did not give rise to a significant increase of analytical signal due to the completion of reactions. A knotted reactor of 200 cm provided sufficient residence time for the development of the color-forming reactions, and it was chosen finally for further assays.

The effect of the flow rate on the on-line generation of the MB dye was studied over the interval 1.3–3.0 ml min^{-1}. The optimal flow rate for maximum response was 1.3 ml min^{-1}. The effect of the carrier flow rate for the loading of the disk phase was investigated over the range 0.9–2.2 ml min^{-1}. Similar trends were observed for both the derivatization reactions and immobilization of MB on the disk phase. The mixing and preconcentration flow rates were thus adjusted at 1.3 and 0.9 ml min^{-1}, respectively, to ensure an optimal analytical signal, while preventing excessive disk flattening and the incomplete sorption of the derivatized product onto the observation zone. Loading flow rates <2.0 ml min^{-1} are typically recommended in optosensing approaches whenever sorption/elution processes are involved [29].

Since the analytical signals are recorded during the retention process, the elution rate is not a critical parameter in solid-phase optosensing. Thus, the elution flow rate was fixed at 5 ml min^{-1}.

Table 2
Analytical performance of the MSFIA-gas-diffusion-optosensing system

Analytical parameter	Value
Detection limit (μg l^{-1})	1.3
Determination limit (μg l^{-1})	4.5
Sensitivity (l μg^{-1})	0.0160 ± 0.0002
Regression coefficient (n = 10)	0.9987 ± 0.0005
Linear working range (μg l^{-1})	20–500
Repeatability (%) (200 μg l^{-1}) (n = 10)	2.2
Reproducibility (%) (100 μg l^{-1}) (n = 10)	6.8
Injection frequency (h^{-1})	5

3.5. Analytical features of the MSFIA-gas diffusion-optosensing method

The analytical features of the optimized MSFIA gas diffusion optosensing procedure are listed in Table 2. A linear working range within one order of magnitude, varying from 20 to 500 μg l^{-1} sulfide, was obtained. Even though diffuse reflectance measurements are characterized for extremely narrow dynamic linear ranges, which are inherent to the Kubelka–Munk function [30] the calibration interval in the present optrode arrangement was particularly wide and comparable to that of spectrophotometric assays [31]. This can be explained by the fact that the analytical signals were treated as apparent absorbance [30] since Lambert–Beer's law was satisfactorily applied for low concentrations of sulfide.

The detection and determination limits were assessed from 3- and 10-times, respectively, the standard deviation of the blank signals. The repeatability was calculated from 10 independent and consecutive measurements at the 200 μg l^{-1} level.

The sensitivity of the proposed approach was calculated as the mean of slopes from 10 day-to-day regression curves.

The robustness of the automatic method was evaluated from the coefficient of variation attained by analyzing a freshly prepared standard (100 μg l^{-1} sulfide) in ten working days using different extraction disks.

Despite the short optical path length of the light beam through the reversed-phase membrane (namely, a mere of few micrometers), the proposed preconcentration methodology improves the detection limit (23-fold lower) and the sensitivity (>350-fold higher) as compared with recently reported MSFIA-GD set-up based on absorbance measurements [24].

3.6. Advantages of the proposed methodology

As compared with different flowing techniques described in the literature for the preconcentration of sulfide using spectrophotometric or reflectometric detection [25,32], MSFIA-GD-optosensing approach provides the best coefficients of variation (2.2% versus 4–5%). This is a consequence of the accuracy of the multisyringe pumps used as automatic liquid drivers as well as to the long-term stability of the light source and the optimum hydrodynamic features of the membrane-based sorbent material.

Besides, it should be stressed that while FIA uses inherently a continuously flowing stream, MSFIA only dispenses well-defined volumes of sample whenever needed. The automatic injection of minute zones of reagents through the activation of solenoid valves also warrants minimum consumption of aggressive reagents (2.8- and 7.7-fold decrease of the amount of HCl and/or H_2SO_4) and chromogenic reagents (37- and 72-fold decrease of DMPD, and 108- and 500-fold reduction of Fe(III)) in comparison with former FIA-manifolds [25,32]. The amount of waste generated is also much lower than that of the other preconcentration methods (1.5- and 2.6-fold diminution).

Although a FIA-reflectometric detection method recently reported features a higher injection throughput [32], the most important drawback derives from the use of C_{18}-bonded silica gel beads, which are packed into the flow-through cell. This is a laborious and time-consuming operational step, which demands the user's skills for the uniform packing of the sensing material as well as for matching the bed layer within the observation field. Moreover, the progressive tighter packing of the sorbent material hinders its long-term unattended applicability. All these shortcomings are readily circumvented by using optrode membranes, which, in turn, assure simple, expeditious, and reliable determinations as demonstrated by the low R.S.D. values obtained.

3.7. Analysis of samples

One of the outstanding advantages of the proposed method with in-line gas-diffusion separation is the possibility of analyzing complex environmental samples, even those containing suspended particulate matter, with no need for neither flocculation nor filtration. The proposed MSFIA-GD-flow-through disk-based solid-phase optosensor was applied to the determination of trace levels of sulfide in groundwater, seawater as well as at the outlet stream of a wastewater treatment plant. Samples were immediately analyzed after collection to prevent oxidation of the target species. The reliability of the method was assessed through recovery studies at concentration levels of 50, 100, 200 and 400 $\mu g\,l^{-1}$ sulfide as shown in Table 3. Recoveries varying within the range 96–115% were obtained for the whole set of samples analyzed indicating that no appreciable oxidative conversion to sulfate occurred during the analyses.

Table 3
Determination of sulfide in environmental waters and wastewaters samples exploiting the MSFIA-gas-diffusion-optosensing hyphenated method

Sample	Added ($\mu g\,l^{-1}$)	Found ($\mu g\,l^{-1}$)	Recovery (%)
Seawater	0	<LOD	–
	50	51 ± 3	102
	200	191.4 ± 0.9	96
	400	392 ± 3	98
Groundwater	0	109 ± 5	–
	50	158.3 ± 0.9	98
	100	219 ± 10	110
	200	313 ± 4	102
Wastewater outlet	0	275.5 ± 0.2	–
	50	333 ± 7	115
	100	380.8 ± 0.9	105
	200	480 ± 7	102

The results are expressed as the mean of three replicates ± standard deviation.

4. Conclusions

The software-controlled multisyringe flow injection system involving the hyphenation of in-line membrane separation with an optical fiber diffuse reflectance sensor for implementing disk-based solid-phase preconcentration provides a robust, selective and sensible method for analyzing untreated complex environmental samples.

The proposed method was satisfactorily applied to sulfide determination in environmental waters and wastewaters. The miniaturized LED-based optical system with isolation and preconcentration capabilities is especially suitable for in-field analysis and real-time monitoring of sulfide, being extensible to other volatile or gas-evolving species of environmental interest by appropriate choice of the LED's and membrane materials. Furthermore, MSFIA affords several additional advantages, such as compactness, versatility and lower sample/reagent consumption, which lead to a minimum waste generation. Besides, this MSFIA set-up is able to perform a large number of analytical operations (namely, sample loading, sample/reagent mixing and rinsing of the flow system) with merely two additional solenoid valves, which also prevent the washing solutions to be pumped through the sorbent micro-zone.

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