

Use of a single air segment to minimise dispersion and improve mixing in sequential injection: turbidimetric determination of sulphate in waters

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Abstract

In this work, we propose the use of an air segment in a sequential injection system to simultaneously improve the overlapping of the stacked zones and minimise dispersion. This strategy was developed for the determination of sulphate in natural and wastewaters. Barium chloride was used as a precipitating agent and the turbidity of the suspension formed was measured at 420 nm. Analysis was performed without sample pre-treatment and the system was able to monitor sulphate concentration at a rate of at least 20 determinations per hour. Slightly different analytical sequences were developed for natural and wastewaters in order to minimise specific interferences. Direct determination of sulphate was possible within a concentration range of 10–100 and 16–100 mg SO₄²⁻ L⁻¹ for natural and wastewaters, respectively. Results obtained were comparable with those of the reference method with relative deviations lower than 5%. Relative standard deviations between 1.6% and 3.3% were found.

1. Introduction

Sequential injection analysis (SIA) has been proposed [1] as an evolution to flow injection analysis [2], presenting the advantages of performing different analyses without system reconfiguration, and a considerable reagent saving associated with non-continuous consumption. These automatic systems are based on the sequential aspiration of well-defined sample and reagent zones into a holding coil (HC), with subsequent flow reversal to propel and mutually disperse these stacked zones through a reaction coil (RC), while heading to the detector. This flow technique has become increasingly

popular among the scientific community and has been successfully applied in the environmental, food, clinical and biotechnological areas. However, when an extensive mixing between the stacked zones is required, either to assure a constant concentration along the whole plug or to provide proper mixing between sample and reagents presenting different physical characteristics, problems might arise. In fact, in sequential injection, mixing is more difficult to achieve than in the flow injection approach, in which solutions are usually efficiently mixed in confluence points. This problem is also overcome using the multicommutation technique with binary sampling proposed by Reis et al. [3], where small plugs of sample and reagents are inserted in an alternate way. All these aspects were discussed by Vieira et al. [4], by testing different sampling approaches in SIA: binary sampling, sandwich sampling and monosegmented flow.

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This last approach was proposed by Pasquini and Oliveira [5], in which sample and reagent are introduced between two air bubbles and removed before they enter the detection system using a gas permeable membrane. This methodology also contributes in overcoming a problem pointed out by Zagatto et al. [6] regarding the fact that measurements in SIA are not taken with maximum reagent concentration, which could increase the extension of interferences.

The novelty of this work is the use of a single air segment in a sequential injection system to simultaneously improve the overlapping of the stacked zones and also reduce dispersion, thus minimising some previously mentioned limitations associated with the sequential injection concept. The air segment is withdrawn through a side port before signal measurement, avoiding the use of a special device for its removal. This strategy was applied to the turbidimetric determination of sulphate in natural and wastewaters, based on the use of barium chloride as precipitating agent and polyvinyl alcohol (PVA) as protective colloid. The same reaction but with different stabilisers (thymol and gelatine) was also used for previously reported sequential injection determinations of sulphate in waters [7–9]. As in this determination the sample must be acidified to prevent anionic interferences, namely in wastewaters, a good mixing between sample and acid plug becomes critical. In order to carry out this process (i.e. to promote sample and reagent overlapping) with minimum sample dispersion, an air segment was introduced just before aspiration of acid, sample and reagent. Additionally, the use of the air segment allowed to separate the stacked zones from the EDTA washing solution, avoiding the use of more complex analytical cycles.

To enable sulphate determination on both types of samples, different experimental conditions were used. For wastewaters, the sample was inserted between two acid plugs and a stop period was used to further extend mixing.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with analytical reagent grade chemicals. To minimise bubble formation inside the flow system, solutions were prepared with previously boiled deionised water. A standard sulphate stock solution (1000 mg L^{-1}), obtained by dissolution of previously dried K_2SO_4 (Riedel-de Haën, Germany), was used to prepare several working standards (in the range $10\text{--}100 \text{ mg L}^{-1}$). A 0.10% (m/v) PVA (Riedel—de Haën, Germany) solution was prepared by suspending 0.5 g of solid in about 200 mL of boiling water with continuous stirring. After cooling, the volume was made

up to 500 mL with deionised water. A 10% (m/v) barium chloride solution was obtained by dissolution of the solid ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ from Merck, Germany) in the 0.10% PVA solution. A buffer solution containing 40 g of EDTA ($\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$ from Merck, Germany), 7 g of ammonium chloride (NH_4Cl from Merck, Germany) and 57 mL of concentrated ammonia (NH_3 from Merck, Germany) was obtained by dissolving the different chemicals in 600 mL of deionised water and diluting to 1 L. Solutions of 0.07 and 0.18 M HNO_3 (Merck, Germany) were also prepared.

2.2. Apparatus

Solutions were propelled by a Gilson Minipuls 3 peristaltic pump (Villiers-le-Bel, France) with a PVC pumping tube. This tube was connected to the central channel of an eight-port electrically actuated selection valve (Valco VICI C15-3118E, Houston, USA).

A Unicam 8625 UV/VIS spectrophotometer (Cambridge, UK) with a Hellma 178.710-QS flow-cell (10 mm light path, $80 \mu\text{L}$ inner volume) (Mülheim/Baden, Germany) was used as the detection system. The wavelength was set at 420 nm. Analytical signals were recorded in a Kipp and Zonen BD 111 strip chart recorder (Delft, Holland). All tubing connecting the different components of the sequential injection system was made of Omnifit PTFE (Cambridge, UK) with 0.8 mm i.d.

A 386 personal computer (Samsung, Korea; SD700) equipped with an Advantec PCL818L interface card, running a homemade software written in QuickBasic 4.5, controlled the selection valve position and the pump rotation direction and speed.

2.3. Sequential injection procedure

The manifold used for the turbidimetric determination of SO_4^{2-} in natural and wastewaters is outlined in Fig. 1. The flow direction and the timing sequence required are listed in Table 1. Flow rate was set at $4.4 \pm 0.1 \text{ mL min}^{-1}$ (calculated as an average of seven determinations).

For natural waters, the EDTA solution, an air segment, 0.07 M HNO_3 solution, sample and BaCl_2 solution were sequentially drawn up into the HC. The flow was then reversed and the volume containing the BaCl_2 solution, the sample and the nitric acid was transferred to the RC. The air segment still remaining in HC was removed through an auxiliary side port. The reaction product was then impelled to the detector and the remaining HC content (EDTA solution and water carrier stream) was flushed to remove any precipitate from the system.

The analytical cycle to determine sulphate in wastewaters had two main differences: (1) addition of an extra

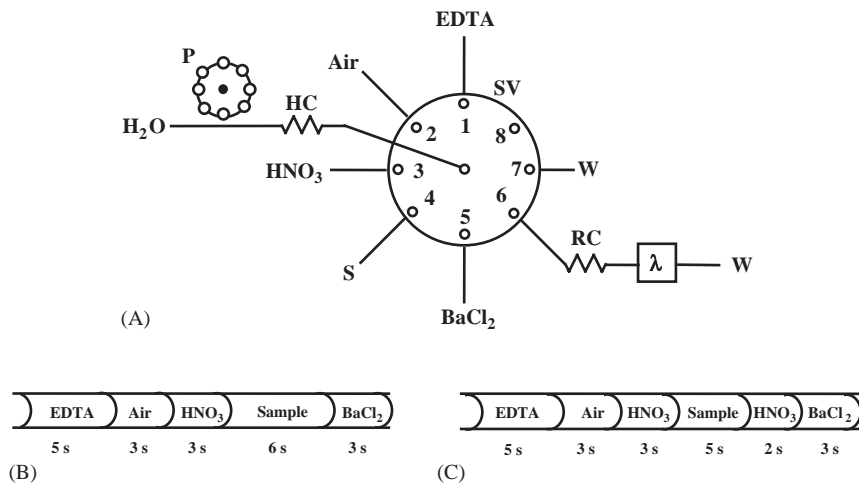


Fig. 1. (A) Sequential injection manifold for the determination of sulphate in natural and wastewaters. SV: selection valve; P: peristaltic pump; HC: holding coil (4.1 m); RC: reaction coil (1.5 m); λ : spectrophotometer (420 nm); W: waste; S: sample or standard; HNO_3 solution: 0.07 and 0.18 M for natural and wastewaters, respectively; and BaCl_2 solution: 10% (m/v) prepared in 0.10% PVA solution; the other connections to the selection valve were 20 cm long. (B and C) Sequence of the solutions in the holding coil, for natural and wastewaters conditions, respectively.

Table 1
Sequential injection protocol sequence for determination of sulphate in waters

Step	Valve position	Operation time (s)	Volume (μL)	Description
A	1	5	367	Draw up EDTA (wash) solution
B	2	3	220	Draw up air segment
C	3	3	220	Draw up HNO_3 solution
D	4	5/6 ^a	367/440 ^a	Draw up sample/standard solutions
E ^b	3	2	147	Draw up HNO_3 solution
F ^b	3	4		Stop period to allow further mixing between sample and acid solution
G	5	3	220	Draw up BaCl_2 solution
H	6	8	587	Pump stack of zones to detector
I	7	8/7 ^a	587/513 ^a	Withdraw air segment
J	6	80	5867	Pump stack of zones to detector, signal registration; system washing

^a Different values for waste and natural waters, respectively.

^b Steps only used for wastewaters.

acid plug (i.e. the sample was inserted between two acid plugs), and (2) use of a stop period to increase mixing between the sample and the acid. Therefore, the analytical sequence was the following: draw up the EDTA solution, an air segment, 0.18 M HNO_3 solution, a plug of sample and 0.18 M HNO_3 solution into the HC. At this point, the flow was stopped by 4 s and then the BaCl_2 solution was drawn up. The subsequent steps were the same as those used in the analytical cycle for natural waters.

2.4. Reference method

In order to assess the quality of the SIA results, they were compared with those provided by the AOAC recommended reference method [10]. In this method, sulphate is also determined turbidimetrically

by precipitation with barium chloride (under controlled acidic and stirring conditions). Turbidity is measured at 420 nm and SO_4^{2-} concentration determined by comparison of the reading with a standard curve (0–40 mg L^{-1}).

3. Results and discussion

3.1. Development of the sequential injection system

In this work, a sequential injection turbidimetric system able to carry out the determination of sulphate in natural and residual waters, without previous treatment, is presented. In this case, sample acidification was imperative to minimise anionic interferences. As sample dilution should also be prevented to enable measurements of low sulphate levels, an air segment was used to

allow the necessary mixing between the sample and nitric acid plugs, without significantly increasing dispersion. This air segment also prevented mixing between the alkaline EDTA and acid solutions.

In a first approach, the same conditions were used for natural and wastewaters. Physical features such as the flow rate and coil dimensions (diameter, length and configuration) were pre-set (Fig. 1), according to a previous SIA system [7]. The analytical sequence was set to: the alkaline EDTA solution, an air segment, nitric acid, sample and BaCl₂ solution (prepared in PVA), which were sequentially drawn up through the selection valve into the HC. The valve was switched to the detector position and the stacked zones (from BaCl₂ solution to the nitric acid solution) propelled to the reactor. At this point, the valve was switched to an auxiliary waste port and the air segment was discarded. Finally, the valve was switched back and the flow headed to the detector.

Using this manifold (Fig. 1), optimisation procedures were then carried out. One of the first features studied was the concentration of the PVA solution used to stabilise the BaSO₄ suspension and thus increase repeatability [11–13]. After using several 10% (m/v) BaCl₂ solutions with different PVA concentrations—0.05, 0.10, 0.25, 0.50% (m/v)—the 0.10% (m/v) was chosen as higher repeatability and sensitivity were obtained.

Regarding barium chloride, concentrations between 5.0% and 15.0% (m/v) BaCl₂ (in 0.10% PVA) solutions were tested. The 10.0% (m/v) BaCl₂ concentration was chosen as sensitivity increased slightly up to this level and then decreased. Additionally, the time to return to baseline significantly increased for higher concentration solutions.

The influence of sample and BaCl₂ solution volumes on sensitivity was also assessed. Different sample volumes—293, 440 and 587 μL—were tested. As expected, the analytical signals were strongly affected, with larger volumes leading to higher turbidity values. The volume of 440 μL was selected based on the fact that the smallest volume provided an unsuitable sensitivity, while the largest volume presented a similar sensitivity value. Regarding BaCl₂ solution, volumes ranging from 220 to 367 μL were used. A volume of 220 μL was selected as a

compromise between sensitivity, reagent consumption and determination throughput. Although peak height increased for larger volumes, no differences were found in the sensitivity, and the sampling rate decreased.

Turbidimetric sulphate procedures are known to be sensitive to pH changes [12,13]. In fact, the reaction kinetic, the extent of interference (from both cationic and anionic species) as well as the structure and solubility of the BaSO₄ precipitate formed are influenced by solution acidity. Therefore, different nitric acid concentrations were tested: 0.035, 0.07 and 0.14 M. The 0.07 M HNO₃ solution was selected as a small increase in sensitivity occurred up to this level and remained constant after that point. No differences were found regarding precision and return to baseline. To assess if this acid concentration level was enough to prevent interference from species usually present in water samples (that might lead to precipitation reactions or change in the acidity of the medium) [13], several 20 mg L⁻¹ sulphate standard solutions with either 500 or 1000 mg L⁻¹ of the interfering species (calcium, hydrogen carbonate, carbonate and magnesium) were prepared. Interference from CO₃²⁻ and HCO₃⁻ was observed. Nitric acid concentration was increased to 0.14 M HNO₃ but the effect of interferences remained. To overcome this problem, a different approach was tested: the sample plug was inserted between two 0.18 M nitric acid plugs. Initially, a sequence of nitric acid–sample–nitric acid, with different sample and nitric acid solution volumes, was used. Nevertheless, interferences persisted, probably due to insufficient overlapping of the plugs. A stop period of 4 s was introduced before aspiration of the BaCl₂ solution. Under these conditions, no interference occurred up to 1000 mg L⁻¹ of CO₃²⁻ and HCO₃⁻. The sample volume was re-evaluated and set to 367 μL. Using this volume no interferences were found. Larger volumes led to no increase in the analysis sensitivity.

After the optimisation studies previously described, a single manifold, with different experimental conditions, was set to determine sulphate in natural and wastewaters. Considering the expected interference levels in natural waters, only one plug of acid appeared to be necessary for the determination. This was confirmed as interferences were only found (Table 2) for carbonate

Table 2
Influence of some interfering species (up to 1000 mg L⁻¹) on the turbidimetric determination of sulphate

Interfering species	Concentration where interference begins (mg L ⁻¹)	
	Natural waters conditions	Wastewaters conditions
Calcium (as CaCl ₂ · 2H ₂ O)	No interference	No interference
Hydrogen carbonate (as NaHCO ₃)	120	No interference
Carbonate (as Na ₂ CO ₃)	100	No interference
Magnesium (as MgCl ₂ · 6H ₂ O)	No interference	No interference

and hydrogen carbonate at concentrations higher than those expected in natural waters. On the other hand, for wastewaters the sample must be inserted between two acid plugs and a stop period must be included to improve mixing. The final conditions for each system are pointed out in Table 1. Under these optimised

conditions, second-order calibration curves were established between 10 and 100 mg SO₄²⁻ L⁻¹ for natural waters, and 16 and 100 mg SO₄²⁻ L⁻¹ for wastewaters.

3.2. Application to water samples

Several natural and wastewaters samples were analysed by the developed flow procedures and by the reference method. The paired results, together with the corresponding relative deviations, are presented in Table 3. A flow register for the determination in wastewaters is shown in Fig. 2.

Table 3
Determination of sulphate (mg L⁻¹) in natural and wastewaters by SIA and by the reference method and corresponding relative deviations (RD)

Sample	Source	SIA ^a	Ref. method	RD (%)
1	Natural	26.5±0.6	27.0	-1.9
2	Natural	27.0±0.5	26.9	+0.4
3	Natural	26.1±0.8	26.4	-1.1
4	Natural	37.1±0.6	37.4	-0.8
5	Natural	11.2±0.5	11.2	0.0
6	Natural	27.5±0.6	27.3	+0.7
7	Natural	26.8±0.7	27.4	-2.2
8	Natural	15.5±0.2	15.4	+0.6
9	Natural	11.4±0.3	11.7	-2.6
10	Natural	25.6±0.6	25.6	0.0
11	Natural	25.6±0.5	25.7	-0.4
12	Waste	72.3±0.4	70.0	+3.3
13	Waste	81.4±1.9	79.6	+2.3
14	Waste	17.7±0.2	18.6	-4.8
15	Waste	36.8±1.2	36.1	+1.9
16	Waste	87.2±1.6	85.8	+1.6
17	Waste	71.3±1.8	74.0	-3.6
18	Waste	27.9±1.2	26.6	+4.9
19	Waste	38.1±0.4	37.5	+1.6
20	Waste	22.3±0.9	22.4	+0.4
21	Waste	23.4±0.9	23.4	0.0

^a Results expressed as the mean of three determinations ±

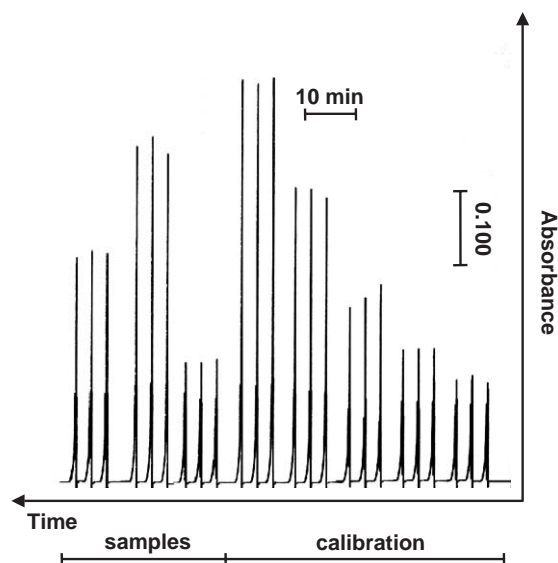


Fig. 2. Recorded output for a set of standards (16, 24, 40, 60 and 100 mg L⁻¹) and wastewater samples.

Table 4
Comparison of results obtained by SIA (C_s) with those of the reference method (C_r)

	Equation parameters (C _s = C ₀ + SC _r)			Characteristics of the SIA system		
	C ₀ (mg L ⁻¹)	S	r ^a	Detection limit (mg L ⁻¹) ^b	RSD (%) ^c	Determination rate (h ⁻¹)
Natural waters	0.036 (±0.608) ^d	0.992 (±0.024) ^d	0.999	10	1.61 (38.0) 2.25 (14.6) 2.55 (25.7)	22
Wastewaters	-0.146 (±2.338) ^d	1.012 (±0.043) ^d	0.999	14	2.22 (24.3) 3.03 (45.7) 3.34 (80.4)	20

^a Correlation coefficient.

^b Detection limit calculated according to IUPAC recommendations [15].

^c Relative standard deviation obtained from 10 consecutive injections of water samples with the respective concentration (mg L⁻¹) indicated in parentheses.

^d Confidence limits for the slope and intercept values, obtained for 95% significance, are indicated in parentheses after the respective values.

To assess the accuracy of flow methodology, a regression of the type $C_S = C_0 + SC_r$ (C_S being the sequential-injection results and C_r those provided by reference method) was established (Table 4). There is a good agreement between the two methodologies as the slope and correlation coefficient are close to unity and intercept values are near zero. Furthermore, confidence limits of the slope and intercept at the 95% confidence level for 8 and 9 degrees of freedom (waste and natural waters, respectively) point out that there is no statistical difference between the two sets of results [14].

The precision of the SIA methods was assessed from 10 consecutive injections of six water samples. Relative standard deviations were lower than 2.6% and 3.4% for natural and wastewaters, respectively (Table 4).

The detection limits were established according to IUPAC recommendations [15]. For natural waters, the detection limit was 10 mg L^{-1} , whereas for wastewaters it was 14 mg L^{-1} .

As the analytical sequences for the two methodologies had some minor changes, the sampling throughputs were also different. Sample frequency was 22 and 20 determinations per hour for natural and wastewaters, respectively.

4. Conclusions

The advantage of using an air segment in a sequential injection system to simultaneously improve the overlapping and minimise the dispersion of the plugs was demonstrated. In this way, one of the main limitations of SIA, namely the one associated with different physical characteristics of sample and reagents, is overcome without requiring a more complex configuration. It should be emphasised that the introduction of an air segment did not affect precision, and was easily removed before measurement without significantly affecting the determination rate.

The developed methodology enabled us to use a single manifold for sulphate determination in natural and wastewaters with good accuracy and precision, and with minor changes in the analytical cycles. These changes mainly regarded the modification of the time over which solutions were drawn up and were promptly controlled in data introduced in the software.

It should also be stressed that in the conditions set for determinations in wastewaters (i.e. inserting the sample between two acid plugs and adding a stop period to allow sufficient mixing), a high level (1000 mg L^{-1}) of interfering species was tolerated. This approach can then be considered as a significant improvement over a previously proposed flow

system [7], in which the authors claim that calcium might have to be removed using a cation-exchange column.

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